CELL CULTURE
CATALOGUE

Classical Media
Special Media
Salt Solutions
Buffers
Water
Reagents
Supplements
Sera
Technical Information

BioConcept

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CELL CULTURE CATALOGUE

BioConcept

BioConcept

Dear Customer

We are proud to present our catalogue, which provides you with a concise overview of our company and our products.

For our catalogue, we have chosen “life in the ocean” as the theme. The oceans were the original incubator of life and provide a complex and delicate balance, which supports the diversity of life. With our cell culture products, BioConcept Ltd. seek to emulate nature by providing the optimal medium to support cell cultivation.

Thank you for using our products and services,

BioConcept Ltd.
Martin Howald
CEO / President

Who we are

BioConcept Ltd. is supplying high-tech tools and products to the biological research community since 1978.

We are a dedicated system supplier, providing our customers with a comprehensive product portfolio and customized solutions.

Going ahead

Over the years we have become a leading supplier and service partner for numerous respected pharmaceutical and academic institutions in Switzerland and around the world.

Iceland

Canada
USA

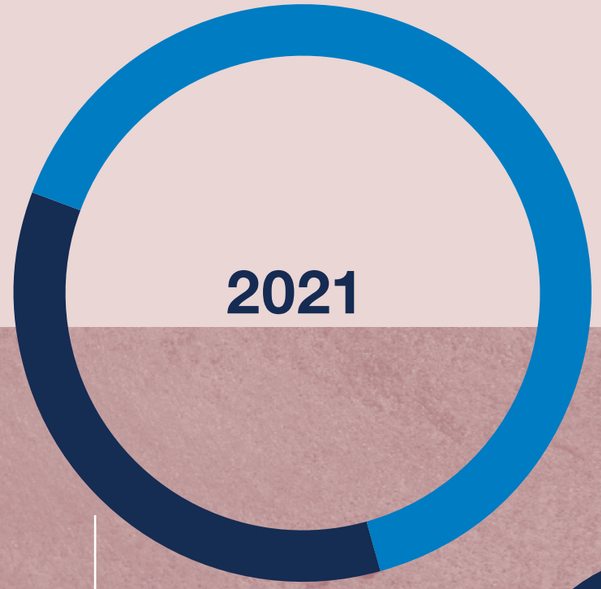
Sub-Saharan Africa

Austria
Czech Republic
Denmark
Estonia
Finland
Germany
Hungary
Italy

Latvia
Lithuania
Netherlands
Poland
Portugal
Slovakia
Sweden

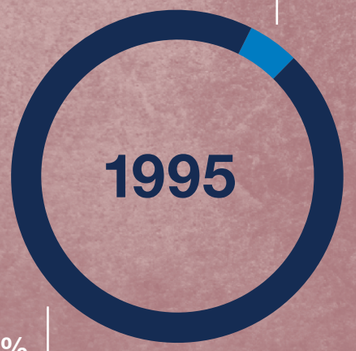
China
India
Japan
Singapore
Taiwan

65%
Sales international



35%
Sales Switzerland

5%
Sales international



95%
Sales Switzerland

With our new facilities, and our convenient location near Basel airport, we aspire to expand further internationally while also serving local biological researchers. We shall continue to listen to the needs of the market and be flexible when meeting them.

1978 Foundation of Flow Laboratories AG

1992 Change of name into BioConcept Ltd. and acquisition of AMIMED Produkte AG

1993 Construction and completion of Liquid I and Powder I

1995 Implementation of the ISO 9001 norm for the whole company

2006 Move to today's location at Paradiesrain 14, Allschwil, Switzerland

2009 Construction of Powder II

2015 Construction of Liquid II

2017 Revision of Liquid I

2019 Expansion of production and office space

Looking back

At BioConcept Ltd. we are proud of the progress we have made since our foundation in 1978.

We have been producing within a certified quality management system since 1995 and our new cell culture and sterile liquid production plant has vastly improved our already high standards. BioConcept Ltd.'s expansion into the tissue culture market in 1992 allowed us to meet the needs of the sophisticated and evolving pharmaceutical and bio-pharmaceutical markets.

Giving back

We believe that we can only operate well in a healthy society and environment.

Hence we commit ourselves to support various cultural and educational programs.

→

Support of the Swiss scientific community

PhD- and postdoctoral retreats aim at fostering scientific exchange and progress. As science no longer recognizes limits and extends beyond various disciplines and geographies, such retreats encourage collaboration between PhD students, postdoctoral Fellows and their mentors.
They will therefore have the opportunity to share their work, discuss and brainstorm science and build new relationships with each other which in turn accelerate the pace of discovery.

Aquaculture & Marine Conservation

Half of the world's population lives along coastlines. Coastal areas have been over-exploited and significantly weakened by climate change. Coral reefs are dying, marine fauna is becoming impoverished, and the stocks of local populations are shrinking. MarineCultures (marinecultures.org) is an organization that sponsors and executes small-scale yet critical conservation projects such as supporting local communities on the coast of Zanzibar to re-establish ecological aquacultures. Together, the community and the authorities, in consultation with experts, carry out critical activities to protect and save marine fauna. Such projects financed by donations and foundations and companies like BioConcept, secure a sustainable livelihood for an increasing number of families on the coast of Zanzibar and contribute to marine life.

Tembea

Tembea refers to the elephant enclosure in the Basel Zoo. With over 5,000 m^2, the new facility is two and a half times the size of the old enclosure. Public can view the resident elephants in their vast and open enclosure very similar to savannah landscape. In addition to African elephants, harvester ants, brown rats and a few species of fish, live in harmony in Tembea which also provides nesting areas for birds and bats. 1

Frauenplus

Frauenplus Baselland is an independent non-profit women's organization dedicated to helping women and their families in difficult circumstances; the organization's objective is to alleviate hardship fast and swiftly.

Isele

In Isele, a small village in East African Tanzania, a new village center is built with help from several sponsors. The new village includes an administration building with offices and a meeting room, a shop and an Internet café. Soon, a small building with workshops and a corn mill will also be housed in the new compound. On the one hand, economic activities are promoted and, on the other hand, existing local communities and social fabric are strengthened. In future, additional buildings will be built in the surrounding area and incorporated into a village structure. 2

Steppin Stompers

"Steppin Stompers" have been an integral part of the Basel area regional jazz scene since 1966. They have performed several hundreds performances and concerts since their debut which is astonishing in and of itself. Their pleasure for playing has never been lost and whomever experiences the Stompers today can hardly imagine that they area band with more than 55 years of cultural contribution under its belt. Time and again, Steppin Stompers like to perform with well known singers and since 1989, the band has afforded itself the luxury of bringing in high caliber guitarists or violinists the likes of Adam Taubitz, a.k.a the devil's violinist, whose nickname is not all an exaggeration of any sort. 3

Florian Schneider

The well-known Swiss singer, actor and songwriter Florian Schneider (Phantom Of The Opera, Heidi, Gotthelf and many more) performs either as a duo or a trio. He is accompanied by the internationally renowned violinist Adam Taubitz (Berlin Philharmonic and many others) and the extremely versatile pianist Roman Bislin. In the very slim line-up with only vocals, guitar, keyboard and violin, it is fascinating to see how incredible the wonderful music connects with soul. 4

Jazz uf em Strich

The annual jazz festival in Sissach, Canton Basel-Landschaft, Switzerland.

Petite Camargue

A few strolls to the north of Basel, nestled between Old Rhine and Grand Canal d'Alsace rivers, lies the largest renaturation site in Europe. The Petite Camargue Alsacienne nature reserve occupies more than nine square kilometers of the Rhine Island and embodies renaturalized areas, ponds, reed beds and open meadows. A University of Basel research station initially conceptualized and still maintains the main concept for renaturalisation that includes Konik horses and Scottish Highland cattle – both of which breeds can survive outdoors all year around – so the mosaic open land and water areas are preserved.
The research station monitors the animals with GPS and conducts scientific studies on the influence of grazing on biodiversity to determine the optimal size of the herd. The station relies on private funding for its research as well as for much of the renaturalization efforts and therefore depends on donations for its existence and operations. BioConcept is a proud sponsor of this wild-life renaturalization effort and wishes that this will result in further recognition of the site as well as more support for similar initiatives.

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Image Florian Schneider >
provided by Kathrin Horn, Eptingen

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About BioConcept Ltd.

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Fish school (sardines)
When sardines (and other forage fish) get threatened instinctively group together and create massive bait balls. Bait balls can be up to 20 metres (66 ft) in diameter. They are short lived, seldom lasting longer than 20 minutes. Fish derive many benefits from shoaling behaviour including defence against predators (by diluting the chance of individual capture), enhanced foraging success, and higher success in finding a mate. A complex combination of senses allows fish to achieve those smooth schooling movements we marvel at. Each fish responds to the movements of the other fish, as well as stimuli such as pheromones. If one fish moves in a different direction all the others sense it and move accordingly.

About us

BioConcept Ltd. is a privately owned company with more than 40 years of experience in serving the biological community. We strive to combine this experience with an innovative spirit to support our customer's needs with the best possible solutions.

To do so, BioConcept Ltd. establishes close relationships with its customers to be able to respond to their wishes. To guarantee that our products are of the highest standard, BioConcept Ltd. has been producing within a certified quality management system (ISO 9001) since 1995. The current ISO standard is 9001:2015. Our SQS certificate can be downloaded at www.bioconcept.ch.

Please contact our specialists if you would like to discuss possibilities in further detail:

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info@bioconcept.ch

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1 The 5000 L state-of-the-art media tank is linked to a cleaning- and sterilization-in-place system (CIP/SIP system).

2 Powder II with a batch size up to 800 kg.

3 Liquid I filling line for special media in the ISO 5 cleanroom.

4 In-house QC (Quality Control) ensures high level of quality.

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4

Infrastructure

BioConcept Ltd. is currently running two independent liquid media production facilities (Liquid I and Liquid II) and two independent powder media production facilities (Powder I and Powder II).

Both are equipped with qualified ISO 5 (GMP A) cleanrooms. Liquid I is used for production of special media and small batch sizes from 5 L up to 1500 L. With Liquid I we are able to handle a broad range of container sizes from 1 ml up to 500 L, giving us a great deal of flexibility to fulfil our customers requests. Liquid II is the fully automated production facility of BioConcept Ltd., which went online in 2015. It is exclusively used for Animal Component Free (ACF) production. The plant has been designed to maximize efficiency, sustainability and productivity. Its layout as well as its equipment is in accordance with GMP guidelines, e.g. air and water preparation facility, calibrated and qualified instruments, validated processes, fully integrated Standard Operating Procedures (SOPs) and comprehensive documentation of production. The state-of-the-art air processing system provides us with the optimal conditions required for sterile liquid production. We constantly monitor and control the pressure, temperature, humidity and particle count in the air and we regularly disinfect our facilities using H_2O_2. The water preparation facilities are specifically engineered to efficiently generate the highest standard Water For Injection (WFI) available. This gives us the capability to generate 5000 L of media a day, allowing us to meet the rising demands from our customers. For the preparation of the bottles we use a cleanroom robot to minimalize the particle count. All further steps, including sealing and labelling, are also performed automatically. In summary, the plant has been designed to create an environment that upholds the highest degree of sterility possible, thereby ensuring a sterile final product.

BioConcept Ltd. runs two powder media plants (Powder I and II) with a capacity of 100 kg/batch and 800 kg/batch, respectively. The impact mills produce powder with an average grain size of < 20 µm. The plants are made for batch sizes from 2 kg up to 800 kg, which can be filled into various container sizes, in accordance to the customers needs. The advantages for the customer of our powder plants are the high homogeneity of the product, with a low particle size, a low risk of cross-contamination and the easy and safe handling.

In addition to these production facilities, BioConcept Ltd. guarantees the high quality of the products with its in-house quality control (QC). BioConcept Ltd. performs tests for sterility, pH-value, osmolality, cell growth, endotoxin levels and conductivity. For powder formulations we also test the bioburden. Further tests, if required, are outsourced to certified analytical laboratories.

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1 The robotic arm plays a crucial role in the automatic filling line in LQII.

2 The automated filling process ensures reliable sterility.

3 In LQII containers up to 500 L are easily handled.

Products

The strength and focus of BioConcept Ltd. lies in manufacturing customized cell culture media as well as defined media for recombinant protein production.

Besides that BioConcept Ltd. offers all standard (classical) media and solutions. Furthermore, the production facilities are superbly equipped to manufacture sterile QC liquids, microbial broths and agars. Therefore, our product line includes:

– Special customer-designed media
– Contract manufacturing of sterile liquids and powder formulations
– Production media for CHO, Hybridoma and Insect Cells
– Individual solutions for your cell culture requirements
– Complete cell systems applications for CHO cells
– Standard media
– Serum-free and ACF media
– Liquid as well as powder media formulations
– Buffers and balanced salt solutions
– Supplements and auxiliary reagents
– Animal sera

In addition to the broad range of cell culture products, we can offer the highest degree of flexibility and customization in a timely manner. Our customers have the following options:

– Modifications of standard products
– New products according to customers recipes
– Outsourcing of media, buffer and WFI production
– Variable batch sizes starting from 5 L up to 5 000 L (liquid) and 2 kg up to 800 kg (powder)
– Variable packaging sizes (1 ml up to 1 000 L) and packaging systems (PET / glass bottles, sterile bags, customized tubing systems, as well as customer specifications)
– Sterilization through sterile filtration (0.22 µm) or hot air/vapour sterilization

For more detailed information please contact us at info@bioconcept.ch.

Classical Media

Basal Medium Eagle with EBS
Dulbecco's MEM (DMEM)
Dulbecco's MEM Low Glucose
Dulbecco's MEM High Glucose
DMEM / Ham's F-12 (1:1)
Ham's F-10 Medium
Ham's F-12 Medium
IMDM
Leibovitz L-15 Medium
Medium 199
McCoy’s Medium
MEM Alpha
MEM EBS
MEM HBS
RPMI 1640
Waymouth's Medium
William's E Medium

Blackeye thicklip [*Hemigymnus melapterus*]
The species is widespread throughout the tropical and subtropical waters. It prefers areas of coral, coral rubble and sand. The Half-and-half wrasse lives solitary or in small groups. It is a benthic predator that feeds mainly on small marine invertebrates such as crustaceans, molluscs, worms and echinoderms captured on or in the substrate. Like most wrasse, it is a protogynous hermaphrodite, i.e. individuals start life as females with the capability of turning male later on.

Garden eel [*Taeniioconger hassi*]

Classical Media

Basal Medium Eagle with EBS

Basal Medium Eagle (BME) was originally developed for studies to determine essential nutrient requirements of mouse “L” cells and HeLa cells in culture. This medium with a host of modifications is widely used and supports both the primary culture of a wide variety of normal mammalian and transformed cell lines. BioConcept Ltd. offers BME liquid with either Earl's Standard formula, Hanks’ salts and/or stable Glutamine.

Basal Medium Eagle

Cat. No.	1-06F01-I BME EBS

Concentration

Inorganic Salts	mg/L
$CaCl_2$ $2H_2O$	265
KCl	400
$MgSO_4$ $7H_2O$	200
NaH_2PO_4 H_2O	140
NaCl	6800
$NaHCO_3$	2220
Other Components	
D(+)-Glucose	1000
Phenol Red	10
Amino Acids	
L-Arginine HCl	21
L-Cystine	12
L-Histidine HCl H_2O	10.81
L-Isoleucine	26
L-Leucine	26
L-Lysine HCl	36.5
L-Methionine	7.5

Amino Acids	mg/L
L-Phenylalanine	16.5
L-Threonine	24
L-Tryptophan	4
L-Tyrosine	18
L-Valine	23.5
Vitamins	
myo-Inositol	1
(+)-Biotin	1
D-Pantothenic acid Calcium salt	1
Choline chloride	1
Folic acid	1
Nicotinamide	1
Pyridoxal HCl	1
Vitamin B2	0.1
Vitamin B1 HCl	1
To be added separately	
L-Glutamine	292

Available Basal Medium Eagle

Cat. No	Modification	Size
1-06F01-I	BME with EBS w/o L-Gln	500 ml
1-06F50-I	BME with EBS, with stable Gln (434 mg/L)	500 ml
1-08F01-I	BME with HBSS, w/o l-Gln	500 ml
1-08F50-I	BME with HBSS, with stable Gln (434 mg/L)	500 ml

Other modifications are available upon request at info@bioconcept.ch.

References

1. Eagle, H. (1955) Proc. Soc. Exp. Biol. Med 89, 362
2. Eagle, H. (1955) J. Biol. Chem. 214, 839
3. Eagle, W.R. (1956) Ann. NY. Acad. Sci. 63, 666
4. Eagle, H. (1959) Science 130, 432

Dulbecco's MEM (DMEM)

Dulbecco's MEM is the most widely used modification of BME. It contains four times the concentration of amino acids and vitamins. Non-essential amino acids and certain essential trace elements were added and the bicarbonate concentration was increased. The standard formula is with 1 000 mg/L glucose, the “high glucose” variation with 4 500 mg/L. Dulbecco’s MEM was originally developed for the culture of mouse embryonic cells. Today it finds a broad application for serum free culture of normal and transformed mouse and chicken cells, e.g. 1:1 with Ham's F-12.

Dulbecco's MEM Low Glucose

Cat. No.	1-25F01-I DMEM Low Glucose
Concentration	
Inorganic Salts	**mg/L**
$CaCl_2$ $2H_2O$	264
$Fe(NO_3)_3$ $9H_2O$	0.1
$MgSO_4$ $7H_2O$	200
KCl	400
NaCl	6 400
NaH_2PO_4 H_2O	125
$NaHCO_3$	3 700
Other Components	
D(+)-Glucose	1 000
Phenol Red	15
Pyruvic acid sodium salt	110
Amino Acids	
Glycine	30
L-Arginine HCl	84
L-Cystine	48
L-Histidine HCl H_2O	42
L-Isoleucine	105
L-Leucine	105
L-Lysine HCl	146

Amino Acids	**mg/L**
L-Methionine	30
L-Phenylalanine	66
L-Serine	42
L-Threonine	95
L-Tryptophan	16
L-Tyrosine	72
L-Valine	94
Vitamins	
Choline chloride	4
D-Pantothenic acid calcium salt	4
Folic acid	4
myo-Inositol	7.2
Nicotinamide	4
Vitamin B1 HCl	4
Vitamin B2	0.4
Vitamin B6 HCl	4
To be added separately	
L-Glutamine	584

Available Dulbecco's MEM Low Glucose Media

Cat. No	Modification	Size
1-25F01-I	DMEM LG w/o L-Gln	500ml
1-25F03-I	DMEM LG with L-Gln	500ml
1-25F04-I	DMEM LG w/o L-Gln, with 25 mM HEPES	500ml
1-25F22-I	DMEM LG w/o L-Gln w/o Phenol Red	500ml
1-25F23-I	DMEM LG (1.0 g/L) with stable Gln (868 mg/L), w/o Phenol Red	500ml
1-25F24-I	DMEM LG w/o L-Gln, with 25 mM HEPES, w/o Phenol Red	500ml
1-25F50-I	DMEM LG with stable Gln (868 mg/L)	500ml
1-25F51-I	DMEM LG with 25 mM HEPES, with stable Gln (868 mg/L)	500ml
1-25P02-K, -L, -M, -N, -W	DMEM LG Powder, with L-Gln	1 L, 5 L, 10 L, 50 L, 100 L
1-25P32-K, -L, -M, -N, -W	DMEM LG Powder, with L-Gln, w/o Phenol Red	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

Dulbecco's MEM High Glucose

Cat. No.	1-26F01-I DMEM High Glucose
Concentration	
Inorganic Salts	**mg/L**
$CaCl_2$ $2H_2O$	264
$Fe(NO_3)_3$ $9H_2O$	0.1
$MgSO_4$ $7H_2O$	200
KCl	400
NaCl	6400
NaH_2PO_4 H_2O	125
$NaHCO_3$	3700
Other Components	
D(+)-Glucose	4500
Phenol Red	15
Amino Acids	
Glycine	30
L-Arginine HCl	84
L-Cystine	48
L-Histidine HCl H_2O	42
L-Isoleucine	105
L-Leucine	105
L-Lysine HCl	146

Amino Acids	**mg/L**
L-Methionine	30
L-Phenylalanine	66
L-Serine	42
L-Threonine	95
L-Tryptophan	16
L-Tyrosine	72
L-Valine	94
Vitamins	
Choline chloride	4
D-Pantothenic acid calcium salt	4
Folic acid	4
myo-Inositol	7.2
Nicotinamide	4
Vitamin B1 HCl	4
Vitamin B2	0.4
Vitamin B6 HCl	4
To be added separately	
L-Glutamine	584

References

1. Dulbecco, R. et al.; (1959) Virology 8, 396
2. Smith, J.D. et al.; (1960) Virology 12, 185
3. Morton, H.J. (1970) In Vitro 6, 89
4. Rutzky, L.P. et al.; (1974) In Vitro 9, 468
5. Ham, R.G. et al.; (1979) Meth.Enzymol. 53, 44
6. Ham, R.G. (1965) Proc.Nat.Acad.Sci. 53, 288
7. Barnes, D. et al.; (1980) Cell 22, 649
8. Barnes, D. et al.; (1980) Anal.Biochem. 102, 255

Available Dulbecco's MEM High Glucose Media

Cat. No	Modification	Size
1-26F01-I	DMEM HG w/o L-Gln	500ml
1-26F03-I	DMEM HG with L-Gln	500ml
1-26F04-I	DMEM HG w/o L-Gln, with 25 mM HEPES	500ml
1-26F22-I	DMEM HG w/o L-Gln w/o Phenol Red	500ml
1-26F23-I	DMEM HG (4.5 g/L) with stable Gln (868 mg/L), w/o Phenol Red	500ml
1-26F50-I	DMEM HG w/o L-Gln, with stable Gln (868 mg/L)	500ml
1-26F51-I	DMEM HG with stable Gln (868 mg/L), with 25 mM HEPES	500ml
1-26F55-I	DMEM HG with 25 mM HEPES, with Sodiumpyruvate (110 mg/L), with stable Gln (868 mg/L)	500ml
1-26F58-I	DMEM High Glucose with Sodiumpyruvate and L-Glutamine	500ml
1-26P02-K, -L, -M, -N, -W	DMEM HG Powder, with L-Gln	1 L, 5 L, 10 L, 50 L, 100 L
1-26P32-K, -L, -M, -N, -W	DMEM HG Powder, with L-Gln, w/o Phenol Red	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

DMEM / Ham's F-12 (1:1)

Cat. No.	1-26F08-I DMEM/F12
Concentration	
Inorganic Salts	**mg/L**
$CaCl_2$ $2H_2O$	154
$CuSO_4$ $5H_2O$	0.00125
Na_2HPO_4 anhydrous	71
$Fe(NO_3)_3$ $9H_2O$	0.05
$FeSO_4$ $7H_2O$	0.417
$MgCl_2$ $6H_2O$	61
$MgSO_4$ $7H_2O$	100
KCl	311.8
NaCl	6999.5
$NaH_2PO_4H_2O$	62.5
$NaHCO_3$	2438
$ZnSO_4$ $7H_2O$	0.43
Other Components	
1,4-Diaminobutane 2 HCl	0.08
D(+)-Glucose	3151
DL-alpha-Lipoic acid	0.105
Hypoxanthine	2.05
Linoleic acid	0.042
Phenol Red	8.1
Pyruvic acid sodium salt	55
Thymidine	0.365
Amino Acids	
Glycine	18.75
L-Alanine	4.45
L-Arginine HCl	147.5
L-Asparagine H_2O	7.5
L-Aspartic acid	6.65
L-Cysteine HCl H_2O	17.56

Amino Acids	**mg/L**
L-Cystine	24
L-Glutamic acid	7.35
L-Histidine HCl H_2O	31.48
L-Isoleucine	54.45
L-Leucine	59.05
L-Lysine HCl	91.25
L-Methionine	17.24
L-Phenylalanine	35.48
L-Proline	17.25
L-Serine	26.25
L-Threonine	53.45
L-Tryptophan	9.02
L-Tyrosine	38.7
L-Valine	52.85
Vitamins	
(+)-Biotin	0.00365
Choline chloride	8.98
D-Pantothenic acid calcium salt	2.24
Folic acid	2.65
myo-Inositol	12.6
Nicotinamide	2.02
Vitamin B1 HCl	2.17
Vitamin B2	0.219
Vitamin B6 HCl	2.031
Vitamin B12	0.68
To be added separately	
L-Glutamine	365

Available DMEM / Ham's F-12 (1:1) Media

Cat. No	Modification	Size
1-26F07-I	DMEM/F-12 w/o L-Gln, with 15 mM HEPES	500ml
1-26F08-I	DMEM/F-12 w/o L-Gln	500ml
1-26F09-I	DMEM/F-12 with stable Gln (543 mg/L)	500ml
1-57F22-I	DMEM/F-12 w/o L-Gln, w/o Phenol Red	500ml
1-57F23-I	DMEM/F-12 with stable Gln (543 mg/L), w/o Phenol Red	500ml
1-57F24-I	DMEM/F-12 w/o L-Gln, with 15 mM HEPES w/o Phenol Red	500ml
1-57F51-I	DMEM/F-12 with 15 mM HEPES, with stable Gln (543 mg/L)	500ml
1-57F54-I	DMEM/F-12 with 15 mM HEPES, with stable Gln (543 mg/L), w/o Phenol Red	500ml
1-57P02-K, -L, -M, -N, -W	DMEM/F-12 Powder with L-Gln	1 L, 5 L, 10 L, 50 L, 100 L
1-57P06-K, -L, -M, -N, -W	DMEM/F-12 Powder with L-Gln, with 15 mM HEPES, w/o Phenol Red	1 L, 5 L, 10 L, 50 L, 100 L
1-57P32-K, -L, -M, -N, -W	DMEM/F-12 Powder with L-Gln, w/o Phenol Red	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

Ham's F-10 Medium

Ham's F-10 Medium is a complex formula containing amino acids, vitamins and certain supplements and ingredients at optimum concentrations. It was originally developed for serum free culture of Chinese Hamster Ovary (CHO) cells, HeLa cell clones and mouse “L” cells. Ham's F-10 is today a widely established and popular medium for the growth of fastidious cell lines with serum or serum free, eg. diploid human cells, white blood cells for chromosomal analysis, primary explants of rat, chicken and rabbit.

Cat. No.	1-13F01-I Ham's F-10
Concentration	
Inorganic Salts	**mg/L**
$CaCl_2$ $2H_2O$	44
$CuSO_4$ $5H_2O$	0.0025
Na_2HPO_4 anhydrous	154
$FeSO_4$ $7H_2O$	0.834
$MgSO_4$ $7H_2O$	153
KCl	285
KH_2PO_4	83
NaCl	7400
$NaHCO_3$	1200
$ZnSO_4$ $7H_2O$	0.0283
Other Components	
D(+)-Glucose	1100
DL-alpha-Lipoic acid	0.21
Hypoxanthine	4.1
Phenol Red	1.2
Pyruvic acid sodium salt	110
Thymidine	0.7
Amino Acids	
Glycine	7.5
L-Alanine	9
L-Arginine HCl	211
L-Asparagine H_2O	15
L-Aspartic acid	13.3
L-Cysteine	25
L-Glutamic acid	14.7
Amino Acids	**mg/L**
L-Histidine HCl H_2O	23
L-Isoleucine	2.6
L-Leucine	13.1
L-Lysine HCl	29
L-Methionine	4.48
L-Phenylalanine	4.96
L-Proline	11.5
L-Serine	10.5
L-Threonine	3.6
Tryptophan	0.6
L-Tyrosine	1.81
L-Valine	3.5
Vitamins	
(+)-Biotin	0.024
Choline chloride	0.7
D-Pantothenic acid Calcium salt	0.715
Folic acid	1.32
myo-Inositol	0.542
Nicotinamide	0.615
Vitamin B1 HCl	1
Vitamin B2	0.375
Vitamin B6 HCl	0.205
Vitamin B12	1.36
To be added separately	
L-Glutamine	146

Available Ham's F-10 Media

Cat. No	Modification	Size
1-13F01-I	Ham's F-10 w/o L-Gln	500 ml
1-13F02-I	Ham's F-10 with L-Gln	500 ml
1-13F04-I	Ham's F-10 w/o L-Gln, with 25 mM HEPES	500 ml
1-13F50-I	Ham's F-10 with stable Gln (217 mg/L)	500 ml

Other modifications are available upon request at info@bioconcept.ch.

References

1. Ham, R.G. (1963) Exp. Cell Res. 29, 515-526
2. Ham, R.G. (1965) Proc. Nat. Acad. Sci. 53, 288-293

Ham's F-12 Medium

Ham's F-12 Medium is a modification of the complex Ham's F-10 formula, containing amino acids, vitamins and trace elements at optimum concentrations. Originally it was developed for serum free culture of Chinese Hamster Ovary (CHO) cells, primary Rat Hepatocyytes and Prostate Epithelial Cells. Ham's F-12 is today the medium of choice for the clonal toxicity assay with CHO-cells (CHD-3 and CHL-1) and supports the growth of a variety of normal, fastidious and transformed cells with serum or serum free.

Cat. No.	1-14F01-I Ham's F-12
Concentration	
Inorganic Salts	**mg/L**
$CaCl_2$ $2H_2O$	44
$CuSO_4$ $5H_2O$	0.0025
Na_2HPO_4 anhydrous	142
$FeSO_4$ $7H_2O$	0.834
$MgCl_2$ $6H_2O$	122
KCl	223.6
NaCl	7599
$NaHCO_3$	1176
$ZnSO_4$ $7H_2O$	0.86
Other Components	
2'-Deoxythymidine	0.73
1,4-Diaminobutane 2 HCl	0.16
D(+)-Glucose	1802
DL-alpha-Lipoic acid	0.21
Hypoxanthine	4.1
Linoleic acid	0.084
Phenol Red	1.2
Pyruvic acid Sodium salt	110
Amino Acids	
Glycine	7.5
L-Alanine	9
L-Arginine HCl	211
L-Asparagine H_2O	15
L-Aspartic acid	13.3
L-Cysteine HCl H_2O	35.12
L-Glutamic acid	14.7

Amino Acids	**mg/L**
L-Histidine HCl H_2O	20.9
L-Isoleucine	3.9
L-Leucine	13.1
L-Lysine HCl	36.5
L-Methionine	4.48
L-Phenylalanine	4.96
L-Proline	34.5
L-Serine	10.5
L-Threonine	11.9
L-Tryptophan	2.04
L-Tyrosine	5.4
L-Valine	11.7
Vitamins	
(+)-Biotin	0.0073
Choline chloride	13.96
D-Pantothenic acid calcium salt	0.48
Folic acid	1.32
myo-Inositol	18
Nicotinamide	0.037
Vitamin B1 HCl	0.34
Vitamin B12	1.36
Vitamin B2	0.038
Vitamin B6 HCl	0.062
To be added separately	
L-Glutamine	146

Available Ham's F-12 Media

Cat. No	Modification	Size
1-14F01-I	Ham's F-12 w/o L-Gln	500 ml
1-14F03-I	Ham's F-12 with L-Gln	500 ml
1-14F04-I	Ham's F-12 w/o L-Gln, with 25 mM HEPES	500 ml
1-14F22-I	Ham's F-12 w/o L-Gln, w/o Phenol Red	500 ml
1-14F50-I	Ham's F-12 with stable Gln (217 mg/L)	500 ml
1-14P02-K, -L, -M, -N, -W	Ham's F-12 Powder with L-Gln	1 L, 5 L, 10 L, 50 L, 100 L
1-14S50-I	Ham's F-12 Kaighn's Modification with stable Gln (434 mg/L)	500 ml

Other modifications are available upon request at info@bioconcept.ch.

References

1. Ham, R.G.; (1963) Exp.Cell.Res. 29, 515
2. Ham, R.G.; (1965) Proc.Nat.Acad.Sci. 53, 288
3. Barnes, D. et al.; (1980) Cell. 22, 649
4. Dulbecco, R. et al.; (1959) Virology, 8 396
5. Smith, J.D. et al.; (1960) Virology 12, 185
6. Morton, H.J.; (1970) In Vitro 6, 89
7. Rutzky, L.P. et al.; (1974) in Vitro 9, 468
8. Ham, R.G. et al.; (1979) Meth. Enzymol. 53, 44
9. Barnes, D. et al.; (1980) Anal.Biochem. 102, 255
10. Kaighn, M.E.; TCMA (1973) 54-58

Iscove's IMDM

Iscove's IMDM, is the most known and widely used modification of Dulbecco's MEM. It is a completely defined, serum free medium, supplemented with albumin, transferrin and selenium, amino acids, vitamins and HEPES buffer, pyruvate and KNO_3 instead of $Fe(NO_3)_3$. Iscove's IMDM was originally developed for rapid propagation of erythropoietic and hemopoietic bone marrow precursor cells in serum free media. It is ideal also for hybridoma and T-/B-Lymphocytes cell culture of both, human and mouse.

Cat. No.	1-28F16-I IMDM w/o Supplements
Concentration	
Inorganic Salts	**mg/L**
$CaCl_2$ $2H_2O$	219
$MgSO_4$ $7H_2O$	200
KCl	330
KNO_3	0.076
NaCl	4505
NaH_2PO_4 $2H_2O$	141
$NaHCO_3$	3024
$NaSeO_3$ $5H_2O$	0.017
Other Components	
D(+)-Glucose	4500
HEPES	5958
Phenol Red	15
Pyruvic acid sodium salt	110
Amino Acids	
Glycine	30
L-Alanine	25
L-Arginine HCl	84
L-Asparagine H_2O	28.4
L-Aspartic acid	30
L-Cystine	70
L-Glutamic acid	75
L-Histidine HCl H_2O	42
L-Isoleucine	105
L-Leucine	105
L-Lysine HCl	146
L-Methionine	30

Amino Acids	mg/L
L-Phenylalanine	66
L-Proline	40
L-Serine	42
L-Threonine	95
L-Tryptophan	16
L-Tyrosine	72
L-Valine	94
Vitamins	
(+)-Biotin	0.013
Choline chloride	4
D-Pantothenic acid calcium salt	4
Folic acid	4
myo-Inositol	7.2
Nicotinamide	4
Vitamin B1 HCl	4
Vitamin B2	0.4
Vitamin B6 HCl	4
Vitamin B12	0.013
To be added separately	
L-Glutamine	584
Iscove's Supplemented Media contain	
BSA	400
Linoleic acid	1
Oleic acid	1
Palmitic acid	1
Transferrin	1

Available Iscove's IMDM Media

Cat. No	Modification	Size
1-28F01-I	I w/o L-Gln, with Supplements	500 ml
1-28F02-I	IMDM with L-Gln, with Supplements	500 ml
1-28F16-I	IMDM w/o L-Gln, w/o Supplements	500 ml
1-28F17-I	IMDM with L-Gln, w/o Supplements	500 ml
1-28F22-I	IMDM w/o L-Gln, w/o Supplements, w/o Phenol Red	500 ml
1-28F50-I	IMDM w/o Supplements, with stable Gln (868 mg/L)	500 ml
1-28P17-K, -L, -M, -N, -W	IMDM Powder, with L-Gln, w/o Supplements	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

References

1. Iscove, N.N. et. al.; (1978) J.Exp.Med. 147, 923
2. Iscove, N.N. et. al.; (1980) Exp.Cell.Res. 126, 121
3. Dulbecco, R. et al.; (1959) Virology, 8, 396
4. Harbour, C. et. al.; (1991) in Mammalian Cell Biotechnology, 1st Ed.; IRL Press, Oxford – NY – Tokyo

Medium 199

Medium 199 is a totally defined nutritive source for explant tissues and cells in cell culture. Properly supplemented with serum, Medium 199 offers broad applicability for cells of different animal species, particularly for the culture of non-transformed cells. It is also used in virology for vaccine production.

Cat. No.	1-21F01-I M 199 EBS

Concentration

Inorganic Salts	mg/L
$CaCl_2$ $2H_2O$	265
$Fe(NO_3)_3$ $9H_2O$	0.72
$MgSO_4$ $7H_2O$	200
KCl	400
NaCl	6800
NaH_2PO_4 H_2O	140
$NaHCO_3$	2200
Other Components	
2-Deoxy-D-ribose	0.5
4-Aminobenzoic acid	0.05
Adenine sulfate	10
AMP H_2O	0.25
ATP disodium salt H_2O	1
Cholesterol	0.2
D(+)-Glucose	1000
D(-)-Ribose	0.5
Guanine HCl	0.3
Hypoxanthine	0.3
L-Glutathione reduced	0.05
Phenol Red	20
CH_3COONa anhydrous	50
Thymine	0.3
Tween 80	20
Uracil	0.3
Xanthine	0.3
Amino Acids	
Glycine	50
L-4-Hydroxyproline	10
L-Alanine	25
L-Arginine HCl	70
L-Aspartic acid	30
L-Cysteine HCL, H_2O	0.11
L-Cystine	20

Amino Acids	mg/L
L-Glutamic acid	66.82
L-Histidine HCL, H_2O	21.88
L-Isoleucine	20
L-Leucine	60
L-Lysine HCl	70
L-Methionine	15
L-Phenylalanine	25
L-Proline	40
L-Serine	25
L-Threonine	30
L-Tryptophan	10
L-Tyrosine	40
L-Valine	25
Vitamins	
(+)-Biotin	0.01
Vitamin K3	0.02
Choline chloride	0.5
D-Pantothenic acid calcium salt	0.01
Folic acid	0.01
myo-Inositol	0.05
Nicotinamide	0.025
Nicotinic acid	0.025
Pyridoxal HCl	0.025
Vitamin B1 HCl	0.01
Vitamin B2	0.01
Vitamin B6 HCl	0.025
Vitamin C	0.05
Vitamin D2	0.1
Vitamin E succinate	0.01
Vitamin A acetate	0.14
To be added separately	
L-Glutamine	100

Cat. No.	1-22F01-I M 199 HBS (Liquid)

Concentration

Amino Acids	mg/L
L-Alanine	25
L-Arginine, HCl	70
L-Aspartic Acid	30
L-Cysteine, HCl H_2O	0.11
L-Cystine	20
L-Glutamic Acid	66.82
Glycin	50
L-Histidine, HCl H_2O	21.88
L-4-Hydroxyproline	10
L-Isoleucine	20
L-Leucine	60
L-Lysine, HCl	70
L-Methionine	15
L-Phenylalanine	25
L-Proline	40
L-Serine	25
L-Threonine	30
L-Tryptophan	10
L-Tyrosin	40
L-Valine	25
Vitamins	
Ascorbic Acid	0.05
Tocopherolsuccinate DL-alfa	0.01
D-Biotin	0.01
Calciferol	0.1
D-Pantothenic Acid (Ca-Salt)	0.01
Choline Chloride	0.5
Folic Acid	0.01
Inositol	0.05
Menadione	0.02
Niacin	0.025
Niacinamide	0.025

Vitamins	mg/L
p-Aminobenzoic Acid	0.05
Pyridoxin, HCl	0.025
Pyridoxal, HCl	0.025
Riboflavin	0.01
Thiamine, HCl	0.01
Vitamin A-acetate	0.14
Inorganic Salts	
$CaCl_2$ $2H_2O$	185.5
NaCl	8000
KCl	400
$Fe(NO_3)_3$ $9H_2O$	0.72
KH_2PO_4	60
$MgSO_4$ $7H_2O$	200
NaH_2PO_4 anhydrous	47.5
$NaHCO_3$	350
Others	**mg/L**
Adenosine 5'-triphosphate 2Na H_2O	1
Adenosine 5'-phosphate H_2O	0.25
Adenine Sulphate	10
L-Glutathione (red.)	0.05
Guanine HCl	0.3
Deoxy-D-ribose	0.5
D-Ribose	0.5
Glucose, D (+)	1000
Hypoxanthine	0.3
Sodium Acetate (anhydr.)	50
Tween 80	20
Xanthine	0.3
Uracil	0.3
Thymine	0.3
Phenol red	20
Cholesterol	0.2

Available Medium 199 Media

Cat. No	Modification	Size
1-21F01-I	M 199 EBS w/o L-Gln	500 ml
1-21F22-I	M 199 EBS w/o L-Gln w/o Phenol Red	500 ml
1-21F50-I	M 199 EBS with stable Gln (149 mg/L)	500 ml
1-22F01-I	M 199 HBS w/o L-Gln	500 ml

Other modifications are available upon request at info@bioconcept.ch.

References

1. Morgan, J.F. et al.; (1950) Proc.Soc.Exp.Biol.Med. 73, 1
2. Morgan, J.F. et al.; (1951) Nat. Canc.Inst. 16/2, 557

McCoy's 5A

McCoy's 5A Medium was created using Basal Medium 5A and is a nutritive substance rich complete medium. McCoy's 5A Medium was originally developed to investigate the amino acid requirements of Novikoff Hepatoma cells. Today this optimal developed medium has a broad application to support the growth of the most demanding primary and continuous cell lines, as well as from biopsy tissues, eg. bone marrow, skin, kidney, omentum, adrenal glands, lung, spleen.

Cat. No.	1-18F01-I McCoy's 5A
Concentration	
Inorganic Salts	**mg/L**
$CaCl_2$ $2H_2O$	132.5
$MgSO_4$ $7H_2O$	200
KCl	400
NaCl	6460
NaH_2PO_4 H_2O	580
$NaHCO_3$	2200
Other Components	
4-Aminobenzoic acid	1
Bacto Peptone	600
D(+)-Glucose	3000
L-Glutathione reduced	0.5
Phenol Red	10
Amino Acids	
Glycine	7.5
L-4-Hydroxyproline	19.7
L-Alanine	13.9
L-Arginine HCl	42.1
L-Asparagine anhydrous	45
L-Aspartic acid	19.97
L-Cysteine	31.5
L-Glutamic acid	22.1
L-Histidine HCl H_2O	20.96
L-Isoleucine	39.36
L-Leucine	39.36
L-Lysine HCl	36.5

Amino Acids	**mg/L**
L-Methionine	14.9
L-Phenylalanine	16.5
L-Proline	17.3
L-Serine	26.3
L-Threonine	17.9
L-Tryptophan	3.1
L-Tyrosine	18.1
L-Valine	17.6
Vitamins	
(+)-Biotin	0.2
Choline chloride	5
D-Pantothenic acid calcium salt	0.2
Folic acid	10
myo-Inositol	36
Nicotinamide	0.5
Nicotinic acid	0.5
Pyriodoxal HCl	0.5
Vitamin B1 HCl	0.2
Vitamin B2	0.2
Vitamin B6 HCl	0.5
Vitamin B12	2
Vitamin C	0.5
To be added separately	
L-Glutamine	219.2

Available McCoy's 5A Media

Cat. No	Modification	Size
1-18F01-I	McCoy's 5A w/o L-Gln	500 ml
1-18F03-I	McCoy's 5A with L-Gln	500 ml
1-18F22-I	McCoy's 5A w/o L-Gln (326 mg/L), w/o Phenol Red	500 ml
1-18F23-I	McCoy's 5A with stable Gln (326 mg/L), w/o Phenol Red	500 ml
1-18F50-I	McCoy's 5A with stable Gln (326 mg/L)	500 ml
1-18F51-I	McCoy's 5A with 25 mM HEPES, with stable Gln (326 mg/L)	500 ml
1-18P02-K, -L, -M, -N, -W	McCoy's 5A Powder, with L-Gln	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

References

1. Neumann, R.E. et al.; (1958) Proc.Soc.Exp.Biol.Med. 98, 303
2. McCoy, T.A. et al.; (1959) Proc.Soc.Exp.Biol.Med. 100, 115
3. Hsu, T.C. et al.; (1960) J.Natl.Canc.Inst. 25, 221
4. Iwakata, S. et al.; (1964) N.Y. State J.Med. 64, 2279
5. Morton, H.J.; (1970) In Vitro 6, 89
6. Park, M.S. et al.; (1974) Transpl. 18, 520
7. Patterson, M.K. et al.; (1978) TCA Manual 4, 737

MEM Alpha

MEM Alpha is a highly complex formulation and is enriched with additional amino acids, vitamins, lipoic acid and pyruvate. It is highly enriched if supplemented with ribo- and desoxyribonucleosides. Originally used for growth of hamster kidney cells, MEM Alpha supports the growth and proliferation of bone marrow and amniotic cells, both in monolayer- and suspension-culture. Supplementation with serum or certain proteins supports the culture. BioConcept Ltd. offers MEM Alpha with and without nucleosides as liquid/powder variations. Modifications are possible on customer requests.

Cat. No.	1-23F01-I

Concentration

Inorganic Salts	mg/L
$CaCl_2$ $2H_2O$	265
$MgSO_4$ $7H_2O$	200
KCl	400
NaCl	6800
NaH_2PO_4 H_2O	140
$NaHCO_3$	2200
Other Components	
D(+)-Glucose	1000
DL-alpha-lipoic acid	0.2
Phenol Red	10
Pyruvic acid sodium salt	110
Amino Acids	
Glycine	50
L-Alanine	25
L-Arginine HCl	127
L-Asparagine H_2O	50
L-Aspartic acid	30
L-Cysteine	100
L-Cystine	24
L-Glutamic acid	75
L-Histidine HCl H_2O	42
L-Isoleucine	52
L-Leucine	52
L-Lysine HCl	73
L-Methionine	15
L-Phenylalanine	32
L-Proline	40
L-Serine	25

Amino Acids	mg/L
L-Threonine	48
L-Tryptophan	10
L-Tyrosine	36
L-Valine	46
Vitamins	
(+)-Biotin	0.1
Choline chloride	1
D-Pantothenic acid calcium salt	1
Folic acid	1
myo-Inositol	2
Nicotinamide	1
Pyriodoxal HCl	1
Vitamin B1 HCl	1
Vitamin B2	0.1
Vitamin B12	1.4
Vitamin C	50
Ribonucleosides	
Adenosine	10
Cytidine	10
Guanosine	10
Uridine	10
Deoxyribonucleosides	
2'-Deoxyadenosine	10
2'-Deoxycytidine HCl	11
2'-Deoxyguanosine H_2O	10.67
2'-Deoxythymidine	10
To be added separately	
L-Glutamine	292

Available MEM Alpha Media

Cat. No	Modification	Size
1-23F01-I	MEM Alpha w/o L-Gln, with Nucleosides	500 ml
1-23F09-I	MEM Alpha w/o L-Gln, w/o Nucleosides w/o Phenol Red	500 ml
1-23S50-I	MEM Alpha with stable Gln (434 mg/L), with Nucleosides	500 ml
1-23F50-I	MEM Alpha with stable Gln (434 mg/L), w/o Nucleosides	500 ml
1-23P10-K, -L, -M, -N, -W	MEM Alpha w/o Nucleosides, Powder	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

References

1. Stanners, C.P. et al.; (1971) Nat.New.Biol. 230, 52
2. Stanners, C.P. et al.; (1975) J.Gen.Virol. 29, 281
3. Earle, W. (1943) J.Natl.Cancer.Inst. 4, 165

MEM EBS and MEM HBS

MEM Eagle, a complex amino acid and vitamin rich medium, was developed from Eagle's Basal Medium (BME). Based on Eagle's Hanks' BSS, MEM is one of the most widely used of all synthetic cell culture media. MEM Eagle with EBS or HBS supports the growth and proliferation of a variety of fastidious primary mammalian cells and established cell lines, both in monolayer- and suspension-culture. Supplementation with serum or certain proteins supports the culture.

Cat. No.	1-31F01-I MEM EBS
Concentration	
Inorganic Salts	**mg/L**
$CaCl_2$ $2H_2O$	267
$MgSO_4$ $7H_2O$	200
KCl	400
NaCl	6800
NaH_2PO_4 H_2O	140
$NaHCO_3$	2200
Other Components	
D(+)-Glucose	1000
Phenol Red	10
Amino Acids	
L-Arginine HCl	126.4
L-Cystine	24
L-Histidine HCl H_2O	41.93
L-Isoleucine	52
L-Leucine	52
L-Lysine HCl	73.06
L-Methionine	14.9
Amino Acids	**mg/L**
L-Phenylalanine	33
L-Threonine	47.6
L-Tryptophan	10.2
L-Tyrosine	36.2
L-Valine	46
Vitamins	
Choline chloride	1
D-Pantothenic acid calcium salt	1
Folic acid	1
myo-Inositol	2
Nicotinamide	1
Pyridoxal HCl	1
Vitamin B1 HCl	1
Vitamin B2	0.1
To be added separately	
L-Glutamine	292

Available MEM EBS Media

Cat. No	Modification	Size
1-31F01-I	MEM EBS w/o L-Gln	500 ml
1-31F03-I	MEM EBS with L-Gln	500 ml
1-31F04-I	MEM EBS w/o L-Gln, with 25 mM HEPES	500 ml
1-31F50-I	MEM EBS with stable Gln (434 mg/L)	500 ml
1-31F51-I	MEM EBS with 25 mM HEPES, with stable Gln (434 mg/L)	500 ml
1-31S01-I	MEM Eagle w/o L-Gln	500 ml
1-31P02-K, -L, -M, -N, -W	MEM EBS Powder with L-Gln	1 L, 5 L, 10 L, 50 L, 100 L
1-31P05-K, -L, -M, -N, -W	MEM EBS Powder, with L-Gln, with 25 mM HEPES	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

References

1. Eagle, H. et al.; (1956) J.Biol.Chem. 214, 845
2. Eagle, H. (1955) Science 122, 501
3. Eagle, H. (1959) Science 130, 432
4. Parker, R.C. (1961) In: Meth.Tis.Cult.; 3rd Ed. Harper NY
5. Eagle, H. (1976) TCA Manual 3, 517
6. Daniel, M.D. et al.; (1969) Proc.Soc.Exp.Biol.Med. 127, 3
7. Ham, R.G. et al.; (1979) Meth.Enzymol. 53, 44
8. Earle, W. (1943) Natl.Cancer.Inst. 4, 165

MEM HBS

Cat. No.	1-33F01-I MEM HBS

Concentration

Inorganic Salts	mg/L
$CaCl_2$ $2H_2O$	185.4
Na_2HPO_4 anhydrous	47.5
$MgSO_4$ $7H_2O$	200
KCl	400
KH_2PO_4	60
NaCl	8000
$NaHCO_3$	350
Other Components	
D(+)-Glucose	1000
Phenol Red	10
Amino Acids	
L-Arginine HCl	126.4
L-Cystine	24
L-Histidine HCl H_2O	41.93
L-Isoleucine	52
L-Leucine	52
L-Lysine HCl	73.06

Amino Acids	mg/L
L-Methionine	14.9
L-Phenylalanine	33
L-Threonine	47.6
L-Tryptophan	10.2
L-Tyrosine	36.2
L-Valine	46
Vitamins	
Choline chloride	1
D-Pantothenic acid calcium salt	1
Folic acid	1
myo-Inositol	2
Nicotinamide	1
Pyridoxal HCl	1
Vitamin B1 HCl	1
Vitamin B2	0.1
To be added separately	
L-Glutamine	292

Available MEM HBS Media

Cat. No	Modification	Size
1-33F01-I	MEM HBS w/o L-Gln	500 ml
1-33F50-I	MEM HBS with stable Gln (434 mg/L)	500 ml
1-33F51-I	MEM HBS with 25 mM HEPES, with stable Gln (434 mg/L)	500 ml

Other modifications are available upon request at info@bioconcept.ch.

References
1. Hanks', J.H.; (1976) TCA Manual 3, 3
2. Hanks', J.H. et al.; (1949) Proc.Soc.Exp.Biol.Med. 71, 19

RPMI 1640

RPMI 1640 was developed as a modification of McCoy's 5A medium and is based on RPMI 1630. RPMI 1640 uses a bi-carbonate buffering system and differs from most mammalian cell culture media in its typical pH 8 formulation. With or without supplemented serum or other defined proteins, RPMI 1640 represents today's most widely applied culture medium for short as well as long term cultivation of many cell types, especially human T/B-lymphocytes (e.g. 72 h PHA stimulation assay), bone marrow cells, hybridoma cells.

Cat. No.	1-41F01-I RPMI 1640

Concentration

Inorganic Salts	mg/L
$Ca(NO_3)_2$ $4H_2O$	100
Na_2HPO_4 anhydrous	800
$MgSO_4$ $7H_2O$	100
KCl	400
NaCl	6000
$NaHCO_3$	2000
Other Components	
4-Aminobenzoic acid	1
D(+)-Glucose	2000
L-Glutathione reduced	1
Phenol Red	5
Amino Acids	
Glycine	10
L-4-Hydroxyproline	20
L-Arginine HCl	241.9
L-Asparagine anhydrous	50
L-Aspartic acid	20
L-Cystine	50
L-Glutamic acid	20
L-Histidine	15
L-Isoleucine	50
L-Leucine	50

Amino Acids	mg/L
L-Lysine HCl	40
L-Methionine	15
L-Phenylalanine	15
L-Proline	20
L-Serine	30
L-Threonine	20
L-Tryptophan	5
L-Tyrosine	20
L-Valine	20
Vitamins	
(+)-Biotin	0.2
Choline chloride	3
D-Pantothenic acid calcium salt	0.25
Folic acid	1
myo-Inositol	35
Nicotinamide	1
Vitamin B1 HCl	1
Vitamin B2	0.2
Vitamin B6 HCl	1
Vitamin B12	0.005
To be added separately	
L-Glutamine	300

Available RPMI 1640 Media

Cat. No	Modification	Size
1-41F01-I	RPMI 1640 w/o L-Gln	500 ml
1-41F03-I	RPMI 1640 with L-Gln	500 ml
1-41F04-I	RPMI 1640 w/o L-Gln with 25 mM HEPES	500 ml
1-41F22-I	RPMI 1640 w/o L-Gln, w/o Phenol Red	500 ml
1-41F23-I	RPMI 1640 with stable Gln (446 mg/L), w/o Phenol Red	500 ml
1-41F24-I	RPMI 1640 w/o L-Gln, with 25 mM HEPES, w/o Phenol Red	500 ml
1-41F50-I	RPMI 1640 with stable Gln (446 mg/L)	500 ml
1-41F51-I	RPMI 1640 with 25 mM HEPES, with stable Gln (446 mg/L)	500 ml
1-41F54-I	RPMI 1640, with 25 mM HEPES, with stable Gln, w/o Phenol Red	500 ml
1-41S07-I	RPMI 1640, w/o L-Gln, w/o Glucose	500 ml
1-41P02-K, -L, -M, -N, -W	RPMI 1640 Powder with L-Gln	1 L, 5 L, 10 L, 50 L, 100 L
1-41P05-K, -L, -M, -N, -W	RPMI 1640 Powder with L-Gln, with 25 mM HEPES	1 L, 5 L, 10 L, 50 L, 100 L
1-41P32-K, -L, -M, -N, -W	RPMI 1640 Powder with L-Gln, w/o Phenol Red	1 L, 5 L, 10 L, 50 L, 100 L

Other modifications are available upon request at info@bioconcept.ch.

References

1. Moore, G.E. et al.; (1967) JAMA 199, 519
2. Moore, G.E. et al.; (1976) TCA Manual, 3, 503
3. Moore, G.E. et al.; (1967) XXI.Ann.Symp.Fund.Canc.Res.Feb., 41
4. Moore, G.E. et al.; (1968) NY.State J.Med. 68, 2054
5. Ham, R.G. et al.; (1979) Meth.Enzymol. 53, 44

Waymouth's Medium

Waymouth's Medium was developed as a serum free, chemically defined synthetic medium for the culture of mouse cell line L929.

Cat. No.	1-46F01-I Waymouth's MB752/1

Concentration

Inorganic Salts	mg/L
$CaCl_2$ $2H_2O$	120
$MgCl_2$ $6H_2O$	240
$MgSO_4$ $7H_2O$	200
KCl	150
NaCl	6000
Na_2HPO_4 anhydrous	300
KH_2PO_4	80
$NaHCO_3$	2240
Other Components	
D(+)-Glucose	5000
Glutathione, reduced	15
Hypoxanthine	25
Phenol Red	10
Amino Acids	
Glycine	50
L-Arginine HCl	75
L-Aspartic acid	60
L-Cysteine HCl H_2O	100.3
L-Cystine	15
L-Glutamic acid	150
L-Histidine HCl H_2O	164.1
L-Isoleucine	25
L-Leucine	50

Amino Acids	mg/L
L-Lysine HCl	240
L-Methionine	50
L-Phenylalanine	50
L-Proline	50
L-Threonine	75
L-Tryptophan	40
L-Tyrosine	40
L-Valine	65
Vitamins	
(+)-Biotin	0.02
Choline chloride	250
D-(Pantothenic acid calcium salt	1
Folic acid	0.5
myo-Inositol	1
Nicotinamide	1
Pyridoxine HCl	1
Vitamin B1 HCl	10
Vitamin B2	1
Vitamin B12	0.20
Vitamin C	17.5
To be added separately	
L-Glutamine	350

Available Waymouth's Media

Cat. No	Modification	Size
1-46F01-I	Waymouth's MB 752/1 w/o L-Gln	500 ml

Other modifications are available upon request at info@bioconcept.ch.

References

1. Waymouth, C.; (1959) J.Nat.Cancer Inst. 22, 1003
2. Waymouth, C.; (1968) Private Communication

William's E Medium

William's E Medium was developed for long term culture of liver epithelial cells of adult rats.

Cat. No.	1-48F01-I William's E Medium

Concentration

Inorganic Salts	mg/L
$CaCl_2$ $2H_2O$	265
$CuSO_4$ $5H_2O$	0.0001
$Fe(NO_3)_3$ $9H_2O$	0.0001
$MgSO_4$ $7H_2O$	200
$MnCl_2$ $4H_2O$	0.0001
KCl	400
NaCl	6800
NaH_2PO_4 $2H_2O$	158
$NaHCO_3$	2200
$ZnSO_4$ $7H_2O$	0.0002
Other Components	
D(+)-Glucose	2000
L-Glutathione reduced	0.05
Methyl linolenate	0.03
Phenol Red	10
Pyruvic acid sodium salt	25
Amino Acids	
Glycine	50
L-Alanine	90
L-Arginine	50
L-Asparagine H_2O	20
L-Aspartic acid	30
L-Cysteine	40
L-Cystine	20
L-Glutamic acid	50
L-Histidine	15
L-Isoleucine	50
L-Leucine	75

Amino Acids	mg/L
L-Lysine HCl	87.6
L-Methionine	15
L-Phenylalanine	25
L-Proline	30
L-Serine	10
L-Threonine	40
L-Tryptophan	10
L-Tyrosine	35
L-Valine	50
Vitamins	
(+)-Biotin	0.5
Vitamin K3	0.01
Choline chloride	1.5
D-Pantothenic acid calcium salt	1
Folic acid	1
myo-Inostol	2
Nicotinamide	1
Pyridoxal HCl	1
Vitamin B1 HCl	1
Vitamin B2	0.1
Vitamin B12	0.2
Vitamin C	2
Vitamin D2	0.1
Vitamin E succinate	0.01
Vitamin A acetate	0.1
To be added separately	
L-Glutamine	292

Available William's E Media

Cat. No	Modification	Size
1-48F01-I	William's E Medium w/o L-Gln	500 ml
1-48F02-I	William's E Medium w/o L-Gln, w/o Phenol Red	500 ml

Other modifications are available upon request at info@bioconcept.ch.

References
1. Williams, G.M. et al.; 1974; Exp.Cell Res. 89, 139
2. Williams, G.M. et al.; 1971; Exp.Cell Res. 69, 106

Special Media

MAM-PF (Production Media)

Hybridoma Express Media

Insect Cell Culture Media

BSK-H Medium for the cultivation of *Borrelia spec.*

SDM 79 for the cultivation of *Trypanosoma spec.*

Stem Cell Media

Clearfin lionfish [*Pterois radiata*]
The Clearfin lionfish also called fireworks fish, is a carnivorous, ray-finned fish with venomous spines that lives in the Indian and western Pacific Oceans. This is the only lionfish species which has spines without any markings. It can also be recognized by the pair of horizontal white stripes on its caudal peduncle. This species is mainly nocturnal. It spends the day hiding in rock crevices, in small caves, or under overhangs. It emerges at night to feed on invertebrates such as crabs and shrimps.

Brain coral [*Physogyra lichtensteini*]

Special Media

MAM-PF® for Serum-Free, Protein-Free and Animal Component Free (ACF) Cell Culture

The breakthroughs in recombinant protein production granted BioConcept Ltd. the ability to produce and supply a new type of cell culture media. BioConcept Ltd.'s MAM-PF® (Mammalian Artificial Media – Protein Free) line of media are tailor made to satisfy the needs of researchers and producers. These media for the cultivation of CHO and BHK cells are fully defined with serum-free, protein-free and ACF (Animal Component Free) versions. ACF media are produced according to EMEA/410/01 all components are certified animal component free – we hold a certificate of origin for every single component.

Products Available

Cat. No.	Description	Size	Serum free	Protein free	Animal Component free (ACF)	Formulation
Mammalian artificial Media – Animal Component free						
10-02F24-I	MAM-PF® 2 growth and production medium w/o Phenol Red	500 ml	×	×	×	Proprietary
10-02F25-I	MAM-PF® 2 growth and production medium with Phenol Red	500 ml	×	×	×	Proprietary
10-02F27-I	MAM-PF® 1 adaptation and growth medium	500 ml	×	×	×	Proprietary
10-02F33-I	MAM-PF® 2 Total Selection Medium (w/o L-Gln, L-Trp, L-His, Gly, Hypoxanthine and Thymidine)	500 ml	×	×	×	Proprietary
10-02S80-I	MAM-PF®77 production medium	500 ml	×	×	×	Proprietary
10-02P74	MAM-PF® 7e Powder Media	flexible	×	×	×	Proprietary
10-02P24 -K,-L,-M,-N,-W	MAM-PF® 2 growth and production medium (powder) w/o Phenol Red, w/o L-Gln, w/o $NaHCO_3$	flexible	×	×	×	Proprietary
5-77F01-H	Lipid-Mixture for MAM-PF® 2 Powder (1 000 ×)	flexible	×	×	×	Proprietary

MAM-PF® Media

Mammalian Artificial Media – Protein and Animal Component Free (ACF) for CHO, BHK, and other mammalian cells

Further development of CHO-Express Media resulted in our MAM-PF® Media Series. These are chemically defined media for the cultivation of a variety of cell lines such as CHO (Chinese Hamster Ovary) Cells or BHK cells and expression of recombinant proteins in suspension cell culture. CHO and BHK cells are the most widely used mammalian cells for the expression of a variety of recombinant proteins.
MAM-PF® Media are formulated without L-glutamine to avoid problems associated with L-glutamine degradation including ammonia accumulation. MAM-PF® Media can be obtained with a minimal amount of Phenol Red or without Phenol Red.

Serum-free culture represents an advance over serum supplemented culture as this facilitates purification and downstream processing of expressed products. Most of the commercially available serum-free media contain various undefined proteins and/or protein hydrolysates. A completely defined formulation is therefore a major advantage over both serum supplemented and serum-free systems. The absence of animal derived proteins is desirable from a regulatory standpoint and also eliminates concerns about consistency and availability. MAM-PF®1, MAM-PF®2, MAM-PF®7e and MAM-PF®77 media are chemically defined media optimized for the growth of Chinese hamster ovary (CHO) or BHK cells and expression of recombinant proteins in suspension culture. MAM-PF® media contain no proteins or peptides of animal origin and no undefined hydrolysates. L-glutamine (0.6–8.00 mM) has to be added by the end user prior to use. Various customers reported best glycosylation patterns of the recombinant expressed product when using MAM-PF® Media.

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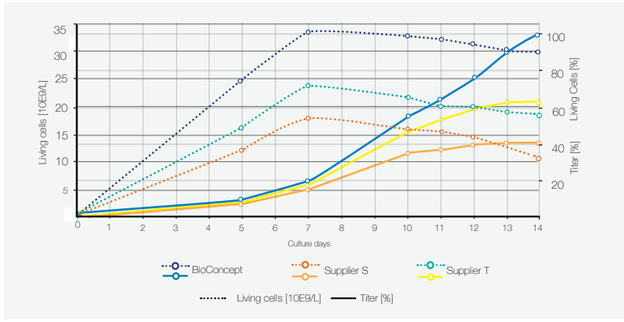
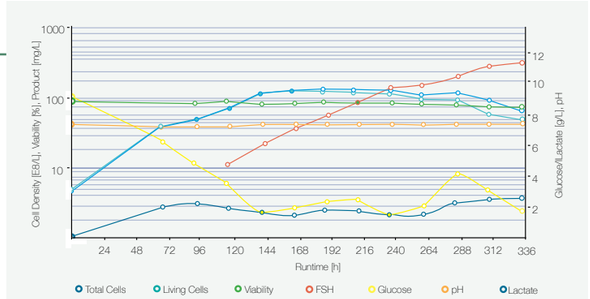
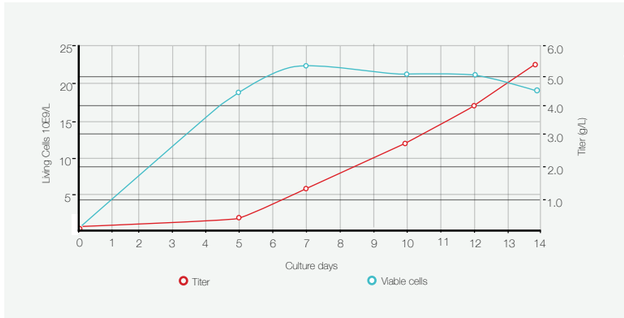


MAM-PF® and other Suppliers
Performance of MAM-PF77® and two different CHO media suppliers in a 14-day fed-batch mAb production.

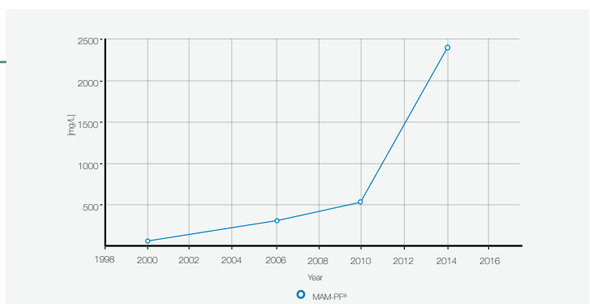
FSH produced with MAM-PF® and FMS3
14-day bioreactor production scheme of the high glycosylated follicle-stimulating hormone (FSH) using MAM-PF77® and the FMS3 feed mix.





Iplimumab produced with MAM-PF® and FSU
High yields of mAbs, e.g. > 5 g/L Iplimumab can be reached in a 14-day fed-batch system using MAMPF77® plus the novel CHO feed mix FMU.

Development of MAM-PF®
Increase of Erythropoietin (EPO) yields during the system development. Within the last 4 years the product yield could be quadrupled up to 2.3 g/L using the MAM-PF77® medium and FMS3 in a fed-batch.



Available MAM-PF® media, supplements and feed mixes

MAM-PF® 1 Liquid, with Phenol Red

Mammalian Artificial Medium – Animal Component Free (ACF)
Intended use: Maintenance of cells, recommended medium for adaptation.
Cat. No. 10-02F27-I, Size: 500 ml, (other sizes – up to 500 L on request)

MAM-PF® 2 Liquid, without Phenol Red

Mammalian Artificial Medium – Animal Component Free (ACF)
Intended use: Batch cultivation to achieve up to 10^7 cells/ml, production medium.
Cat. No. 10-02F24-I, Size: 500 ml, (other sizes – up to 500 L on request)

MAM-PF® 2 Powder, without Phenol Red

Mammalian Artificial Medium – Animal Component Free (ACF)
Intended use: Batch cultivation to achieve up to 10^7 cells/ml, production medium.
Cat. No. 10-02P24-K, -L, -M, -N, -W, Sizes: 1 L, 5 L, 10 L, 50 L, 100 L (other sizes on request)

MAM-PF® 2 powder is formulated without $NaHCO_3$. It is supplied with a sterile supplement (Lipid Mix Cat. No. 5-77F01).

MAM-PF® 77 Liquid, without Phenol Red

Mammalian Artificial Medium – Animal Component Free (ACF)
Intended use: Batch cultivation to achieve up to 4×10^7 cells/ml, production medium.
Cat. No. 10-02S80-I, Size: 500 ml, (other sizes – up to 500 L on request)

MAM-PF® 7e Powder, without Phenol Red

Mammalian Artificial Medium – Animal Component Free (ACF)
Intended use: Batch cultivation to achieve up to 4×10^7 cells/ml, production medium.
Cat. No. 10-02P74, various quantities are available on request
The protocol for reconstitution of 10-02P74 is shown on **page 114.**

Supplementary Products

CHO-Lipid Mix (1 000×)

Intended use: CHO Lipid mixture is a 1 000× concentrate which boosts cell culture of CHO-Cells in suspension. Animal Component Free formulation (proprietary), can also be used in feeding strategies.
Cat. No. 5-74S08-H, Size: 100 ml (other sizes on request)

Lipid Mix for MAM-PF®2 powder (1 000×)

Intended use: This lipid mixture is needed as a supplement to reconstitute MAM-PF®2 powder to liquid. Animal Component Free formulation (proprietary).
Cat. No. 5-77F01-H, Size: 100 ml (other sizes on request)

CHO-Feed Mixes

MAM-PF® 6 Supplement

Intended use: Concentrate used in feeding strategies. Animal Component Free formulation (proprietary) of amino acids, vitamins and other components, no hydrolysates.
Cat. No. 5-14K06-I, Size: 500 ml (other sizes on request)

CHO-Feed-Mix-Powder U1 37

Cat. No. 5-09215-GB, Size 684.4g (4 L) (other sizes on request).
Recommended feed usage: Feed mix for CHO cells

CHO Feed Mix Powder U2 B13

Cat. No. 5-0916-AV, 49.6g (1 L) other sizes on request.
Recommended feed usage: Feed mix for CHO cells

CHO-Feed Mix U1beta7

Cat. No. 5-09Z15
Sizes: 648.4 g (5-09Z15-GB), 5 kg (5-09Z15-UB), 10 kg (5-09Z15-UC)
(Customized packaging on request)

Reconstitution: use 162.1 g powder for 1 L Medium
1. Dissolve 162.1 g in 950 ml 50 °C H_2O (double distilled or equivalently purified).
2. Use 19 ml 10 M NaOH to reconstitute 1 L Feed Mix. Add H_2O ad 1 L.
3. NaOH-solution may be replaced by NaOH tablets if necessary.
4. Final pH after reconstitution: 7.0 – 7.60. Perform sterile filtration (0.22 µm).

CHO-Feed Mix U2beta13

Cat. No. 5-09Z16
Sizes: 49.6 g (5-09Z15-AV), 100 g (5-09Z16-O), 1 kg (5-09Z16-U)
(Customized packaging on request)

Reconstitution: use 49.6 g powder for 1 L Medium
1. Dissolve 49.6 g in 950 ml H_2O (double distilled or equivalently purified). Add 14.8 g
2. NaOH tablets. Add H_2O ad 1 L. NaOH tablets may be replaced by dissolved NaOH if necessary.
3. Final pH after reconstitution: ~ 10.5 – 11.5. Perform sterile filtration (0.22 µm).

Usage: U2beta13 (5mL/feed*L) + U1beta7 (20mL/feed*L)

Trace Element Mixes

Intended use: Substitution of media lacking inorganic components.
Animal Component Free formulation.

Available formulations
Cat. No. 9-00K78-H/-I/-K
Cat. No. 9-00K64-H/-I/-K/-AC
Size: 100 ml/500 ml/1 L/2 L (other sizes on request)

9-00K78	9-00K64
Components	**Components**
$CdCl_2 \cdot 2H_2O$	$NiSO_4 \cdot 6H_2O$
$ZnSO_4 \cdot 7H_2O$	NH_4VO_3
$NaVO_3$	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$
$CoCl_2 \cdot 6H_2O$	$Na_2SiO_3 \cdot 5H_2O$
$CuSO4 \cdot 5H_2O$	$MnSO_4 \cdot H_2O$
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	$C_6H_5FeO_7 \cdot H_2O$
$BaCl_2 \cdot 2H_2O$	$ZnSO_4 \cdot 7H_2O$
$MnSO_4 \cdot H_2O$	$CuSO_4 \cdot 5H_2O$
$SnCl_2 \cdot 2H_2O$	$SnCl_2 \cdot 2H_2O$
$NiSO_4 \cdot 6H_2O$	
KI	
KBr	
$CrK(SO_4)_2 \cdot 12H_2O$	
NaF	
$AgNO_3$	
RbCl	
$ZrOCl_2 \cdot 8H_2O$	
$AlCl_3 \cdot 6H_2O$	
GeO_2	
$TiCl_4$	
$Na_2SiO_3 \cdot 5H_2O$	

Phenol Red (1.5 g/L)

Most MAM-PF® media are formulated without Phenol Red. If Phenol Red needs to be added: Cat. No. 5-70F01-H, Size 100 ml, Formulation: 1.5 g/L, sterile

Express Media for Hybridoma Cells

HYGM 6 and 7 Express Media
Hybridoma Growth Media (HYGM) 6 and 7 are serum free and fully defined media that can be used for the cultivation of various different hybridomas and the production of monoclonal antibodies. Both media are available with or without phenol red to prevent interference of the dye with chromogenic assays.
HYGM-6 and 7 media are ready to use.

HYGM-6 Medium is a serum free medium and contains recombinant insulin for further manufacturing use. It is the only protein in that medium, no other proteins or undefined hydrolysates are present. HYGM-7 Medium is a chemically defined medium and does not contain any undefined hydrolysates or proteins.

ISF-1 Serum-free medium for Hybridoma cell culture
ISF-1 is a serum-free, chemically defined medium for hybridoma cell culture and monoclonal antibody production. It contains Glutamine and does not require further supplementation. To protect cells from shear forces during production, surfactant is included in the medium. ISF-1 is not suitable for cholesterol dependent cell lines (e.g. NSO and its varients), without further supplementation of lipopprotein.
The BSA used in ISF-1 is EDQM-certified. The addition of antibiotics should not be a substitute for proper sterile techniques. Therefore, use of antibiotics is in most cases neither necessary nor advised. However, in those instances where antibiotics are desired, ISF-1 has been shown to be compatible with the most used antibiotics (e.g. gentamycin, puromycin and amphotericin B).

Cat. No.	Description	Size	Serum free	Protein free	Animal Component free (ACF)	Formulation
Express Media for Hybridoma cells						
9-00F55-I	HYGM-6 Express, with phenol red	500 ml	×			Proprietary
9-00F57-I	HYGM-6 Express, w/o phenol red	500 ml	×			Proprietary
9-00F58-I	HYGM-7 Express, w/o phenol red	500 ml	×	×	×	Proprietary
9-00F67-I	HYGM-7 Express, with phenol red	500 ml	×	×	×	Proprietary
1-57S97-I	ISF-1 Hybridoma Growth Medium	500 ml	×			Proprietary

For technical information and protocols please check **page 114**

Insect Cell Culture Media

General information
Nutrient requirements of insects are generally similar to those of vertebrates, but there are also some remarkable exceptions which have to be implemented when designing insect cell culture media.

Steroids
As insects have no capacity for steroidogenesis, insect cell culture media need a source for formation of cell membrane components and the steroid hormone ecdysone (Law and Wells, 1989).

Amino acids
Insect blood contains a high level of amino acids. Media for insect cell culture therefore contains high levels of amino acids (Grace 1962, Weiss et al. 1981).

Organic acids
Insect blood contains an unusually high level of free organic acids such as citrate, succinate, oxalate and malate which range from 0.1–30 mmoles per insect (Grace 1962, Vaughn 1968).

pH, buffer and pH indicator
Insect tissue fluids are more acidic and normally ranging from 6.2–6.9 (Grace 1962). Optimal range for most insect cell culture media is therefore between 6.2–6.5 compared to 7.1–7.6 for most mammalian cell culture media. SF-3 Baculo Express is optimised to keep this range under various culture conditions (e.g. open air, open capped). Insect cell culture media are buffered with sodium phosphate, no CO_2 is required to keep a constant range. No pH indicator is added to insect cell culture media. The colour of insect media is therefore yellow due to the supplementation of protein hydrolysates. In SF-3 Baculo Express these hydrolysates are added as ultrafiltrates with an 8 kDa cut off to facilitate downstream purification processes.

Osmolality
Osmotic pressure varies significantly from that in vertebrate blood, being more than twice as high (Vaughn 1968). Insect cell culture media therefore exhibit a osmolality of 340–390 mOsmol/kg compared to 290–330 mOsmol/kg for vertebrate cultures.

Glutamine / Glucose
The excess metabolism of glutamine and glucose in mammalian cell culture results in an excess production of ammonium and lactate respectively (Ljunggren and Häggström, 1992), and accumulation of these metabolic byproducts is often inhibitory. Detoxification of these metabolites in insect cells follows a different pathway than in mammalian cells. Therefore higher levels of Glutamine and Glucose can be used in insect cell culture media to support high cell densities growth (reviewed in: Schläger, 1996).

Available Insect Cell Culture Media

Cat. No.	Description	Size	Serum free	Protein free	Formulation
9-00F38-I	SF-4 Baculo Express ICM «ready to use»	500 ml	×	Contains yeast extract as only undefined component	Proprietary
9-00F38-K	SF-4 Baculo Express ICM «ready to use»	1 L	×	Contains yeast extract as only undefined component	Proprietary
9-07S38-I	SF-4 Baculo Express (1.1 × conc.) w/o yeast extract, w/o L-Valine	500 ml	×	×	Proprietary
9-10S38-I	SF-4 Baculo Express (1.1 × conc.) w/o yeast extract, w/o L-Methionine	500 ml	×	×	Proprietary
9-05S38-I	SF-4 Baculo Express (1.1 × conc.) w/o yeast extract, w/o L-Tyrosine	500 ml	×	×	Proprietary
9-02S38-I	SF-4 Baculo Express (1.1 × conc.) w/o yeast extract, w/o amino acids	500 ml	×	×	Proprietary
1-43F00-I	Schneider's *Drosophila* Medium w/o L-Gln	500 ml		×	See page 55
1-43F02-I	Schneider's *Drosophila* Medium with L-Gln	500 ml		×	See page 55
1-34F00-I	Mitsuhashi and Maramorosh	500 ml	Contains Lactalbumin-hydrolsate and yeast extract		See page 54

Other modifications of SF-4 Media are available at info@bioconcept.ch.

SF-Baculo Express Media

Extensive further development and further investigation on the nutritional needs of insect cells, based on the excellent performance of SF-1, resulted in our new improved “Ready to Use” insect medium. Already successfully used in different academic and industrial laboratories, SF-4 show following improvements:

1. Cell density: Densities up to 2×10^7 cells/ml could be achieved using SF-4 in bioreactors and spinner flasks
2. Versatility: Not only suitable for SF9 and SF21 but also High Five TM and *Drosophila* cells
3. Adaptation: Only few passages are needed, if you switch from your current serum supplemented medium (e.g. TC-100 or Grace's). Direct switch from your current serum free (but not protein free) medium is possible for some of the commercially available media.
4. Protein yield: Results indicate an increased protein yield (1.5–2.7 times) in recombinant protein production compared to previously used media.

SF-4 Baculo Express ICM (Insect Culture Medium)
9-00F38, Ready to use, no supplementation required. Formulation proprietary.

SF-4 Baculo express medium is a proprietary formulation which has successfully been used to grow various *Spodoptera frugiperda* (SF9, SF21) , BTI-TN-5B1-4 (High Five™) and *Drosophila melanogaster* (D.Mel-2) cells.

Amino acid depleted SF-4 medium (Cat. No. 9-02S38-I, 9-05S38-I, 9-10S38-I) is an efficient reagent for isotope labeling in NMR studies (see References **page 53**).

Other modifications are available upon request at info@bioconcept.ch.

For technical information and protocols please check page 116.

References

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Mitsuhashi / Maramorosh Insect Medium

Mitsuhashi and Maramorosh basal medium is suited for mosquito cell culture. The complete medium has to be supplemented with FCS prior to use.

Mitsuhashi / Maramorosh Insect Medium	1-34F00-I

Concentration

Inorganic Salts	mg/L
$CaCl_2$ $2H_2O$	200
KCl	200
$MgCl_2$ $6H_2O$	100
NaCl	7000
$NaHCO_3$	120
NaH_2PO_4 H_2O	200

Other Components	mg/L
D-Glucose	4000
Lactalbumin Hydrolysate	6500
Yeastolate	5000

References

1. Mitsuhashi, J. (1982) Media for Insect Cell Cultures. In: Advances in Cell Culture vol. 2, K. Maramorosh ed., Academic Press, N.Y., pp. 133–196
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Schneider's *Drosophila* Medium

This Medium was originally developed for the culture of *Drosophila* cells. However, it can also be used for the culture of other dipteran cell lines.

Schneider's *Drosophila* Medium	1-43F00-I

Concentration

Inorganic Salts	mg/L
$CaCl_2$ $2H_2O$	795
KCl	1600
KH_2PO_4	450
$MgSO_4$ $7H_2O$	3700
NaCl	2100
$NaHCO_3$	400
Na_2HPO_4	700
Other Components	
α-Ketoglutaric	200
Fumaric acid	100
D-Glucose	2000
L(-)-Malic acid	100
Succinic acid	100
Trehalose	2000
Yeastolate	2000
Amino Acids	
α-Alanin	500
L-Arginine	400
L-Aspartic Acid	400

Amino Acids	mg/L
L-Cysteine	60
L-Cystine	100
L-Glutamic Acid	800
Glycine	250
L-Histidine	400
L-Isoleucine	150
L-Leucine	150
L-Lysine HCl	1650
L-Methionine	800
L-Phenylalanine	150
L-Proline	1700
L-Serine	250
L-Threonine	350
L-Tryptophan	100
L-Tyrosine	500
L-Valine	300
To be added separately	
L-Glutamine	1800

Cat. No. 1-43F02-I contains L-Glutamine.

References

1. Schneider, Imogene (1964) Exp. Zool. 156: 1:91
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BSK-H Medium for the Cultivation of *Borrelia* Spec.

BSK-H medium is a high quality nutrient liquid for the reliable cultivation of *Borrelia* spec. BSK-H medium is a quite complex formulation with an extraordinary high content of proteins and peptides. It is especially rich in nucleosides, glucose as energy source and contains high concentrations of vitamins. The medium contains N-Acetyl-D-Glucosamine which is an essential element of the bacterial peptidoglycan.

Only selected reagents of highest quality are used for the production of BSK-H medium. A special galenic as well as a gentle and controlled process guarantee a product of highest quality with reliable lot to lot constancy, stability, purity and reliable cell growth. A sufficient high amount of HEPES guarantees a stable buffer capacity for a long time. The concentration of CO_2 as well as the pH value have to be controlled carefully, because in some cases, due to metabolisation of glucose in long-term cultures, lactic acid accumulation occurs, which may result in a reduction of the pH-value of more than 1 pH unit. At room temperature the pH value is 7.6 ± 0.2 at an osmolality of 420 ± 20 mOsm/kg H_2O. The ready-to-use medium has to be supplemented with 3 – 8 % rabbit serum prior to inoculation. Storage temperature and stability are according to lot-specific label.

BSK-H medium can be widely used for the cultivation of spirochetes, especially for *B. burgdorferi* and *B. hermsii*. Only small amounts of organisms are sufficient for the inoculation. However, the values found in the literature, as well as personal communications are varying too much, so that no general recommendation for the inoculum number can be given. The generation time lies between 11 and 18 hours, so that in 7 to 9 days $0.5 – 4.0 \times 10^8$ cells/ml can be obtained. The optimal incubation temperature lies between 30 °C and 37 °C.

Formulation of BSK-H Medium

BSK-H Medium Borrelia — **1-10S03-I**

Concentration

Inorganic Salts	mg/L
$CaCl_2$ $2H_2O$	264.92
KCl	400
$MgSO_4$ $7H_2O$	200
NaCl	6800
$NaHCO_3$	2200
NaH_2PO_4 H_2O	140
Other Components	
N-Acetyl-D-Glucosanmine	400
Cocarboxylase $4H_2O$	1
Coenzyme A $3H_2O$	2.5
Albumin	50000
Neopeptone	5000
Yestolate	2000
D-Glucose	6000
Glutathione Reduced	10
Sodium Acetate anhydrous	50
Sodium Glucuronate	4.2
Citric Acid H_2O	500
Pyruvic Acid	800
HEPES	6000
Tween 80	5
Phenol Red	20
Amino Acids	
L-Alanine	25
L-Arginine HCl	70
L-Aspartic Acid	30
L-Cysteine HCl H_2O	260
L-Cystine	20
L-Glutamic Acid	75
Glycine	50
L-Histidine HCl H_2O	20
Trans-4-Hydroxy-L-Proline	10
L-Isoleucine	20
L-Leucine	60
L-Lysine HCl	70
L-Methionine	15

Amino Acids	mg/L
L-Phenylalanine	25
L-Proline	40
L-Serine	25
L-Threonine	30
L-Tryptophan	10
L-Tyrosine	40
L-Valine	25
Vitamins	
Asorbic Acid	50
Biotin	0.01
D-Ca Pantothenate	0.01
Cholesterol	0.20
Choline Chloride	0.50
Folic Acid	0.01
i-Inositol	0.05
Niacinamide	0.025
Niacin	0.025
Paraaminobenzoic Acid	0.05
Pyridoxal HCl	0.025
Pyridoxin HCl	0.025
Riboflavin	0.01
Thiamine	0.01
Nucleosides / Nucleotides	
Deoxyadenosine	10
Deoxycytidine HCl	11.6
Deoxyguanosine	10
Thymidine	10
5-Methyldeoxycytidine	0.1
FlavinAdenine Dinucleotide	1
NAD	7
β-NADP 2Na	1
Uridine-5Triphosphate 3Na	1
To be added separately	
Rabbit Serum	3 – 8 %
To be added optionally	
L-Glutamine	100 – 300*

*BSK-H Medum with 300 mg/L L-Glutamine, Cat. No. 1-10S02-I, Size: 500 ml

Note:
Prior to inoculation with bacteria, medium and serum should be heated to 30 – 32 °C.

SDM 79 For the Cultivation of *Trypanosoma* Spec.

Brun and Jenni (1979) developed a semi-defined medium for *Trypanosoma brucei*, which has successfully been used to culture various species of *Trypanosoma*. This medium is available in both liquid and powder.

Cat. No.	Description	Size
9-03V02-K, -L, -M, -N	SM Medium (Powder)*	1 L, 5 L, 10 L, 50 L
9-04S22-I	SDM79 (liquid)	500 ml
9-04V01-K, -L, -M, -N	SDM79 (Powder)	1 L, 5 L, 10 L, 50 L

*SM Medium for *T. Brucei procyclic* forms (formulation according to Cunningham)

Formulation of SDM79

Concentration

Inorganic Salts	mg/L
$CaCl_2$ anhyd.	189.21
$Fe(NO_3)_3$ $9H_2O$	0.15
KCl	378.43
$MgSO_4$ anhyd.	92.39
NaCl	6433.26
$NaHCO_3$	2000
$NaH2PO_4$ H_2O	132.45
Other Components	
D (+) Glucosamine HCl	50
Taurine	180
D-Ribose	0.105
Hypoxanthine	0.063
Desoxy-D-Ribose	0.105
Tween 80	4.19
D-Glucose	1946.07
Glutathione Reduced	0.01
Sodium Acetate anhyd.	10.48
Sodium Pyruvate	100
HEPES	8000
MOPS	5000
Phenol Red	11.56
Amino Acids	
L-Alanine	210.58
L-Arginine HCl	229.20
L-Asparagine H_2O	9
L-Aspartic Acid	14.27
L-Cysteine HCl H_2O	0.023
L-Cystine 2HCl	28.63
L-Glutamine	536.01
L-Glutamic Acid	22.82
Glycine	14.98
L-Histidine HCl H_2O	35.63
Trans-4-Hydroxy-L-Proline	2.10
L-Isoleucine	46.89
L-Leucine	51.08
L-Lysine HCl	68.77
L-Methionine	84.18

Amino Acids	mg/L
L-Phenylalanine	109.67
L-Proline	615.28
L-Threonine	391.53
L-Serine	71.54
L-Tryptophan	9.65
L-Tyrosine 2 Na-Salt	169.70
L-Valine	39.30
Vitamins	
Asorbic Acid	0.01
Thiamine HCl	0.74
Biotin	0.202
D-Ca Pantothenate	0.74
Cholesterol	0.042
Choline Chloride	0.84
Folic Acid	4.74
i-Inositol	1.483
Niacinamide	0.742
Niacin	0.005
Paraaminobenzoic Acid	2.01
Pyridoxal HCl	0.742
Pyridoxin HCl	0.0052
Riboflavin	0.076
DL-alpha-Tocopherol succinate	0.0021
Ergocalciferol	0.021
Vitamin A Acetate	0.030
Menadion	0.0021
Nucleosides and Precursers	
Adenosine	10
Adenine Sulfate	2.096
Guanosine	10
Guanine HCl	0.063
Xanthine	0.063
Uracil	0.063
Thymin	0.063
AMP H_2O	0.052
ATP 2Na H_2O	0.21 BIT 9500 Serum Substitute

References

Brun R. and Schonenberger M. (1979) Cultivation and in vitro cloning of procyclic culture forms of Trypanosoma brucei in a semi-defined medium. Acta Trop,36:289–92.

BIT 9500 Serum Substitute

Serum is a highly variable non-standardized product requiring extensive testing to find the batch compatible with your cell types. The composition of serum-free medium on the other hand is well defined and ensures lot-to-lot consistency. BIT 9500 Serum Substitute replaces serum for the culture of stem cells and was developed for use in serum-free culture conditions with defined composition. BIT 9500 contains Bovine serum albumin (BSA), Insulin and Transferrin (BIT) in Iscove's IMDM. For BSA we use tested batches of EDQM certified Probumin™ BSA.

Cell Types: serum-free culturing of Stem Cells in general and Hematopoietic Progenitor, Pluripotent Stem Cells, Hybridomas, Peripheral Blood Mononuclear Cells (PBMC's), Mast Cell lines in particular amongst others

Species:
human and mouse

Product Properties:
– pH is set at 7.3 ± 0.2 and osmolality at 300 ± 20 mOsm/kg/H_2O
– Store below -15°C
– Contains Bovine serum albumin, recombinant human insulin, human transferrin (iron-saturated), Iscove's IMDM
– Not intended for any human or animal diagnostic or therapeutic use

Instructions for use:
Thaw at room temperature (15–20°C) or overnight at 2–8°C. Swirl the bottle to mix its content. Store at 2–8°C for up to 1 month. Alternatively, aliquot and store at -20°C. After thawing the aliquots, do not refreeze. Use BIT 9500 at a final concentration of 20% in your preferred medium.

Cat. No.	Description	Size
5-18S02-H	BIT 9500 Serum Substitute	100 ml

Salt Solutions / Buffers / Water / Reagents & Supplements

Salt Solutions / Buffers

Water

Media Supplements

Reagents

Trypsin Solutions

Antibiotic / Antimycotic Solutions

Moon jellyfish [*Aurelia aurita*]
The moon jellyfish, or moonjelly, is found throughout the world's oceans. Around the size of a plate, it is recognisable by the four circles visible through the translucent white bell. These four circles are gonads, the reproductive organs located at the bottom of the stomach, and they are normally purple in colour. Moonjellies have short, delicate tentacles that hang down from the sides of the bell. They catch their plankton prey using a layer of mucus over their bells before passing the microscopic meals into their mouth parts using special tentacles.

Pulse coral or Xenia soft coral [*Xenia spec.*]

Salt Solutions / Buffers

Earle's Balanced Salt Solution EBSS

Cat. No.	3-01F29-I
Unit Size	500 ml
Presentation	1 × liquid
Formula	w/o Ca^{++}/Mg^{++}
Concentration	
Inorganic Salts	**g/L**
$CaCl_2$ $2H_2O$	–
KCl	0.40
NaCl	6.80
$NaHCO_3$	2.20
NaH_2PO_4 H_2O	0.14
Other Components	
D-Glucose	1.00
Phenol Red	0.01

Hanks' Balanced Salt Solution (HBSS)

Cat. No.	3-02F00-I	3-02F31-I	3-02F29-I	3-02F33-I
Unit Size	500 ml	500 ml	500 ml	500 ml
Presentation	1 × liquid	1 × liquid	1 × liquid	1 × liquid
Formula	with Ca^{++}/Mg^{++}	w/o Phenol Red	w/o Ca^{++}/Mg^{++}	w/o Ca^{++}/Mg^{++} w/o Phenol Red
Concentration				
Inorganic Salts	g/L	g/L	g/L	g/L
$CaCl_2\ 2H_2O$	0.185	0.185	–	–
KCl	0.40	0.40	0.40	0.40
KH_2PO_4	0.06	0.06	0.06	0.06
$MgSO_4$	0.20	0.20	–	–
NaCl	8.00	8.00	8.00	8.00
$NaHCO_3$	0.35	0.35	0.35	0.35
Na_2HPO_4	0.048	0.048	0.048	0.048
Other Components				
D-Glucose	1.00	1.00	1.00	1.00
Phenol Red	0.01	–	0.01	–

Cat. No.	3-02K32-I
Unit Size	500 ml
Presentation	10 × liquid
Formula	w/o Phenol Red
Concentration	
Inorganic Salts	g/L
$CaCl_2\ 2H_2O$	1.85
KCl	4.00
KH_2PO_4	0.60
$MgSO_4\ 7H_2O$	2.00
NaCl	80.00
Na_2HPO_4	0.48
Other Components	
D-Glucose	10.00
Phenol Red	–
To be added separately	
$NaHCO_3$	3.50

Phosphate Buffered Saline (pH 7.4)

Cat. No.	3-05F39-I
Unit Size	500 ml
Presentation	1 × liquid
Concentration	
Inorganic Salts	g/L
KH_2PO_4	0.144
$Na_2HPO_4\ 7H_2O$	0.795
NaCl	9.00

Physiological (0.9%) Sodium Chloride Solution

Cat. No.	3-06S00-I
Unit Size	500 ml
Presentation	1 × liquid
Concentration	
Inorganic Salts	g/L
NaCl	90.00

Dulbecco's PBS

Cat. No.	3-05F00-I	3-05F29-I/-K	3-05K00-I	3-05K29-I
Unit Size	500 ml	500 ml, 1 L	500 ml	500 ml
Presentation	1 × liquid	1 × liquid	10 × liquid	10 × liquid
Formula	with Ca^{++}/Mg^{++}	w/o Ca^{++}/Mg^{++}	Standard	w/o Ca^{++}/Mg^{++}
Concentration				
Inorganic Salts	**g/L**	**g/L**	**g/L**	**g/L**
$CaCl_2$ $2H_2O$	0.132	–	1.32	–
KCl	0.20	0.20	2.00	2.00
KH_2PO_4	0.20	0.20	2.00	2.00
$MgCl_2$ $6H_2O$	0.10	–	1.00	–
NaCl	8.00	8.00	80.00	80.00
Na_2HPO_4	1.15	1.15	11.50	11.50

Cat. No.	8-05F00-I	8-05F29-I	3-05P00 -K, -L, -M, -N	3-05P29 -K, -L, -M, -N
Unit Size	500 ml	500 ml	1L, 5L, 10L, 50L	1L, 5L, 10L, 50L
Presentation	1 × liquid	1 × liquid	powder	powder
Formula	with Ca^{++}/Mg^{++} Endotoxin free	w/o Ca^{++}/Mg^{++} Endotoxin free	Standard	w/o Ca^{++}/Mg^{++}
Concentration				
Inorganic Salts	**g/L**	**g/L**	**g/L**	**g/L**
$CaCl_2$ $2H_2O$	0.132	–	–	–
KCl	0.20	0.20	0.20	0.20
KH_2PO_4	0.20	0.20	0.20	0.20
$MgCl_2$ $6H_2O$	0.10	–	0.10	–
NaCl	8.00	8.00	8.00	8.00
Na_2HPO_4	1.15	1.15	–	–
To be added seperately				
$CaCl_2$ anhydrous	–	–	0.1	–

EDTA PBS (0.02%)

Cat. No.	5-32F00-H/-I
Unit Size	100 ml / 500 ml
Presentation	0.02% solution
Formula	w/o Ca^{++}/Mg^{++}

Sodium Bicarbonate Solution

Cat. No.	5-30F00-H/-I
Unit Size	100 ml / 500 ml
Presentation	7.5% solution
Formula	75 g/L

HEPES Buffer

Cat. No.	5-31F00-H
Unit Size	100 ml
Presentation	1 M solution
Formula	238.3 g/L

HEPES Powder (Free Acid)

Cat. No.	5-31P00-O/-R
Unit Size	100 g / 500 g
Presentation	powder
Formula	free acid

Red Blood Cell (RBC) Lysis Buffer

Cat. No.	3-13F00-H
Unit Size	100 ml
Presentation	liquid
Formula	Standard

Water

Water is a key component for many cell biology and molecular biology applications as well as the manufacturing of liquid biopharmaceutical medium. Because of this we have invested heavily into our water purification facilities. The water preparation facilities were carefully engineered for optimum quality assurance, efficiency, and sustainability and are able to quickly and effectively generate the highest standard Water for Injection (WFI) available. Furthermore, quality has not been sacrificed to improve the quantity; at BioConcept we now produce up to 5000 L/day with our liquid media plant, which allows us to meet the rising demands from our customers.

Water for Injection (WFI), sterile

Water for Injection (WFI) is high quality water for use as a solvent in the preparation of media and laboratory reagents. Available in many sizes to meet your needs. The water is prepared by electronic deionisation, distillation and sterile filtration (0.1 µm), with no added substances. We monitor our water quality on a routine basis. Conductivity, total organic carbon, endotoxin and bioburden and metals are tested weekly. In addition each Batch is tested for sterility, endotoxin, conductivity, pH, osmolality, and oxidizable substances. BioConcept WFI exceeds the European Pharmacopeia specifications (EP) and is produced according to cGMP.

Cat. No.	8-00F33-I
Unit Size	500 ml other units upon request
Presentation	liquid
Formula	WFI according to current EP

Applications:
BioConcept WFI Water is intended for manufacturing or research use.
Not for diagnostic or therapeutic use.

Applications for WFI Water include:
– Hydration of cell culture media, supplements, and salt solutions
– Rinsing of primary packaging, production vessels, equipment, and room surfaces
– Reconstitution and rehydration of product during synthesis
– Preparation of rinse, purification, and chromatography buffers

Water for Cell Culture (Endotoxin Free), sterile

Cat. No.	8-73F00-I/-K/-M
Unit Size	500 ml / 1 L / 10 L
Presentation	liquid
Formula	Endotoxin free <0.01 EU/ml

Information
Water for cell culture is an endotoxin-free and sterile high-quality water. It is suitable for the use as solvent in the preparation of cell culture media, buffers and supplements as well as many more applications in the field of tissue culture.

Water for Molecular Biology, sterile

Cat. No.	3-07F04-H, -I
Unit Size	100 ml / 500 ml
Presentation	liquid
Formula	DNA/DNAse RNAse free, PCR Inhibitor free

Information
Water for molecular biology is sterile and highly pure water free of DNA, DNase, RNase and PCR inhibitors. It is ideal for a wide range of molecular biology applications such as PCR, DNA sequencing, DNA/RNA/protein extraction and purification. It is not DEPC-treated

LAL Reagent Water, sterile

Cat. No.	8-07F22-I
Unit Size	500 ml
Presentation	liquid
Formula	Endotoxin free <0.005 EU/ml

Information
LAL Water is used for preparation of any control or sample when performing an endotoxin detection test. LAL Water is equivalent to Water for Bacterial Endotoxins Test (BET). LAL water is tested to purity requirements beyond most other non-pyrogenic water products. LAL Water is tested to ensure that the endotoxin levels are < 0.005 EU/mL, which is needed to perform an endotoxin assay. This Endotoxin level is fifty times lower than the USP/EP WFI requirement which is below 0.250 EU/ml.

Media Supplements

MEM Amino Acids Solution

Cat. No.	5-12K01-H
Unit Size	100 ml
Presentation	50 × liquid
Formula	w/o Glutamine

Concentration Amino Acids	g/L
L-Arginine HCl	6.32
L-Cystine	1.20
L-Histidine HCl H_2O	2.10
L-Isoleucine	2.625
L-Leucine	2.62
L-Lysine HCl	3.625
L-Methionine	0.755
L-Phenylalanine	1.65
L-Threonine	2.38
L-Tryptophan	0.51
L-Tyrosine	1.80
L-Valine	2.34

MEM Non-Essential (NE) Amino Acids Solution

Cat. No.	5-13K00-H
Unit Size	100 ml
Presentation	100 × liquid
Formula	Standard

Concentration Amino Acids	g/L
L-Alanine	0.89
L-Asparagine H_2O	1.50
L-Aspartic Acid	1.33
L-Glutamic Acid	1.47
Glycine	0.75
L-Proline	1.15
L-Serine	1.05

L-Glutamine Solution

Cat. No.	5-10K00-H
Unit Size	100 ml
Presentation	100 × liquid
Formula	200 mM (28.2 g/L) L-Gln

L-Glutamine Powder

Cat. No.	5-10P00-O/-R
Unit Size	100 g/500 g
Presentation	powder

Stable Glutamine Solution

Cat. No.	5-10K50-H
Unit Size	100 ml
Presentation	100 × liquid
Formula	200 mM (43.44 g/L) L-Ala-L-Gln

Stable Glutamine Powder

Cat. No.	CN-38-O/-R
Unit Size	100 g/500 g
Presentation	powder

Diamond Vitamin Tween™ 80 Solution (40×)

Cat. No.	5-78F00-I
Unit Size	500 ml
Presentation	40× liquid

Concentration

Components	mg/L
Menadion (Vitamin K3)	11
(+) -a-Tocopherolacetate	5
Ergocalciferol (Vit D2)	55
Vitamin A Acetate	55
DL-a-Lipoic Acid	6.7
D-Biotin	5.5
D-Calcium Pantothenate	5.5
Choline Chloride	276.7
Cyanoccobalamin (Vitamin B12)	4
Folic Acid	5.5
i-Inositol	27.7
Niacin	10.6
Niacinamide	10.6
4-Aminobenzoic Acid (PABA, Vit H1) 27.7	27.7
Pyridoxal HCl	10.6
Pyrodoxin HCl	10.6
Riboflavin (Vitamin B2)	5.5
Thiamin HCl (Vitamin B1 HCl)	5.5
Tween™ 80	4445
Ethanol p.A.	70.06 ml

Tween™ is a registered trademark of CRODA International Plc.

CHO Lipid Mix

Cat. No.	5-74S08-E/-H
Unit Size	20 ml/100 ml
Presentation	liquid
Formula	1 000× (Proprietary)

Lutrol® F-68

Cat. No.	5-75F02-H
Unit Size	100 ml
Presentation	liquid
Formula	10% solution

Transferrin (Human, holo)

Cat. No.	5-67F00-D/-G
Unit Size	100 ml/50 ml
Presentation	liquid
Formula	4 mg/ml

BSA Solution

Cat. No.	5-66F00-H
Unit Size	100 ml
Presentation	liquid
Formula	100 mg/ml

2-Mercaptoethanol

Cat. No.	5-69F00-E
Unit Size	20 ml
Presentation	1 000× liquid
Formula	50 mM

Nucleosid Mix (100×)

Cat. No.	5-82F00-I/-K/-H
Unit Size	100 ml/500 ml/1 L
Presentation	100× liquid
Formula	Standard

Concentration

Components	mg/L
Cytidine	1 000
Guanosine	1 000
Uridine	1 000
Adenosine	1 000
2' Deoxyadenosine	1 000
2' Deoxycytidine HCl	1 100
2' Deoxyguanosine H_2O	1 067
2' Deoxythymidine	1 000

Sodium Pyruvate

Cat. No.	5-60F00-H
Unit Size	100 ml
Presentation	100 mM solution
Formula	11 g/L

Insulin (Human, Recombinant)

Cat. No.	5-79F00-G
Unit Size	50 ml
Presentation	liquid
Formula	5 mg/ml

Phenol Red Solution

Cat. No.	5-70F01-H
Unit Size	100 ml
Presentation	liquid
Formula	1.5 g/L

HT-Supplement

Cat. No.	5-64K00-H
Unit Size	100 ml
Presentation	50 × liquid
Formula	Standard
Concentration	
Components	**g/L**
Hypoxanthine	680
Thymidine	193
NaCl	9000

HAT-Supplement

Cat. No.	5-63K00-H
Unit Size	100 ml
Presentation	50 × liquid
Formula	Standard
Concentration	
Components	**mg/L**
Hypoxanthine	680
Aminopterin	8
Thymidine	193
NaCl	9000

Glucose Solution (50%)

Cat. No.	5-33F01-H/-I
Unit Size	100 ml / 500 ml
Presentation	liquid
Formula	Standard
Concentration	
Components	**g/L**
Glucose	500

Amino Acids CC grade (Powder)

Amino acid	Product Code (100g)	Product Code (500g)
L-Alanine	CN-9002-O	C-9001-R
L-Arginine	CN-9003-O	C-9003-R
L-Asparagine H_2O	CN-9006-O	C-9006-R
L-Aspartic Acid	CN-9007-O	C-9007-R
L-Cystine	CN-9009-O	C-9009-R
L-Glutamine	5-10P00-O	5-10P00-R
L-Glutamic Acid	CN-9013-O	C-9013-R
Glycin	CN-9014-O	C-9014-R
L-Histidine	CN-9015-O	C-9015-R
L-Isoleucine	CN-9019-O	C-9019-R
L-Leucine	CN-9020-O	C-9020-R
L-Lysine HCl	CN-9022-O	C-9022-R
L-Methionine	CN-9023-O	C-9023-R
L-Phenylalanine	CN-9024-O	C-9024-R
L-Proline	CN-9025-O	C-9025-R
L-Serine	CN-9026-O	C-9026-R
L-Threonine	CN-9027-O	C-9027-R
L-Tryptophan	CN-9029-O	C-9029-R
L-Tyrosine	CN-9030-O	C-9030-R
L-Valine	CN-9031-O	C-9031-R

Methylcellulose

Cat. No.	9-00F14-I
Unit Size	500 ml
Presentation	0.5% liquid
Formula	Endotoxin free, cell culture grade

Information
Methylcellulose is used as a carrier substrate for test substances that have to be injected into animals. Methylcellulose increases the viscosity of the cell culture medium and thereby protects the cell from mechanical stresses and fluid movements in the culture dish.

Trypan Blue

Cat. No.	5-72F00-H
Unit Size	100 ml
Presentation	0.5% liquid, not sterile
Formula	5 g/L in normal saline

Information
Trypan Blue is a stain recommended for use in dye exlcusion procedures to count viable cells. Viable cells do not take up the dye whereas dead cells do.

Reagents

20 × SSC und 10 × TBE

Cat. No.	3-07F00-I	3-07F01-I
Unit Size	500 ml	500 ml
Presentation (liquid)	20 × SSC	10 × TBE
Formula	according to Maniatis	according to Maniatis
Concentration		
Components	**g/L**	**g/L**
Tri-Sodiumcitrate $2H_2O$	88.23	–
NaCl	175.32	–
Tris (hydroxymethyl) aminomethane	–	121.14
Boric acid	–	55.647
EDTA 2Na $2H_2O$	–	37.224

SDS Solution (20%)

Cat. No.	3-07F02-I
Unit Size	500 ml
Presentation	20% solution
Formula	20% SDS

Tris-Acetate-EDTA (TAE) buffer, 50x

Cat. No.	3-07F03-I
Unit Size	500 ml
Presentation	50 × liquid
Formula	Tris 2M
	Acetic Acid 1M
	Disodium EDTA 50 mM

Trypsin Solutions

Trypsin-EDTA (0.05%), w/o Ca^{++}/Mg^{++}, with Phenol red

Cat. No.	5-51F00-H/-I
Unit Size	100 ml / 500 ml
Presentation	1 × liquid
Formula	EDTA PBS 1:250 (0.05%/0.02%) w/o Ca^{++}/Mg^{++} with Phenol Red

Trypsin-EDTA (0.25%), w/o Ca^{++}/Mg^{++}, w/o Phenol red

Cat. No.	5-52F00-H/-I
Unit Size	100 ml / 500 ml
Presentation	1 × liquid
Formula	EDTA PBS 1:250 (0.25%/0.02%) w/o Ca^{++}/Mg^{++} w/o Phenol Red

Trypsin-EDTA (0.05%) w/o Ca^{++}/Mg^{++}, w/o Phenol red

Cat. No.	5-53F00-H
Unit Size	100 ml
Presentation	1 × liquid
Formula	EDTA PBS 1:250 (0.05%/0.02%) Ca^{++}/Mg^{++} w/o Phenol Red

Trypsin-EDTA (0.25%), w/o Ca^{++}/Mg^{++}, with Phenol red

Cat. No.	5-54F00-H
Unit Size	100 ml
Presentation	1 × liquid
Formula	EDTA PBS 1:250 (0.25%/0.02%) w/o Ca^{++}/Mg^{++} with Phenol Red

Trypsin-EDTA (0.5%) 10x, w/o Ca^{++}/Mg^{++}, with Phenol red

Cat. No.	5-51K00-H
Unit Size	100 ml
Presentation	10 × liquid
Formula	EDTA PBS 1:250 (10 ×) (0.5%/0.2%) w/o Ca^{++}/Mg^{++} with Phenol Red

Antibiotic / Antimycotic Solutions

PSN (Pen-Strep-Neomycin)

Cat. No.	4-13F00-H
Unit Size	100 ml
Presentation / ml	5 mg / 5 mg / 10 mg
Spectrum	$Gram^+$ / $Gram^-$

Geneticin® G418 50 mg/ml

Cat. No.	4-15F01-H/-E
Unit Size	100 ml / 20 ml

Used as a selective agent in mammalian cells at concentrations ranging from 400 to 1 000 µg/ml. G418 blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic cells. Resistance to G418 is conferred by the Neomycin resistance gene (neo) from Tn5 encoding an aminoglycoside 3'-phosphotransferase, APH(3')-II.

Geneticin® G418 crystalline

Cat. No.	4-15P01-GA
Unit Size	5 mg

Used as a selective agent in mammalian cells at concentrations ranging from 400 to 1 000 µg/ml. G418 blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic cells. Resistance to G418 is conferred by the Neomycin resistance gene (neo) from Tn5 encoding an aminoglycoside 3'-phosphotransferase, APH(3')-II.

Hygromycin B 50 mg/ml

Cat. No.	4-21F01-E
Unit Size	20 ml

Used as a selective agent in molecular genetics experiments on a wide variety of eukaryotic and prokaryotic species. The hph gene confers hygromycin-resistance to cells expressing it. Mammalian cells are sensitive to Hygromycin B concentrations of 50–200 µg/ml, and bacteria to 50–100 µg/ml.

Amphotericin B (Fungizone®)

Cat. No.	4-05F00-H
Unit Size	100 ml
Presentation / ml	250 µg
Suggested Working Conc.	2.5 µg/ml
Spectrum	Fungi / Yeasts

Penicillin / Streptomycin / Fungizone®

Cat. No.	4-02F00-H
Unit Size	100 ml
Presentation / ml	10000 IU / 10000 µg / 25 µg
Suggested Working Conc.	1:100
Spectrum	Bacteria, Fungi, Yeasts

Penicillin

Cat. No.	4-12F00-H
Unit Size	100 ml
Presentation / ml	10000 IU
Suggested Working Conc.	1:100
Spectrum	$Gram^+$

Penicillin / Streptomycin

Cat. No.	4-01F00-H
Unit Size	100 ml
Presentation / ml	10000 IU / 10000 µg
Suggested Working Conc.	1:100
Spectrum	$Gram^+$ / $Gram^-$

Gentamicin

Cat. No.	4-07F00-H
Unit Size	100 ml
Presentation / ml	5000 µg
Suggested Working Conc.	50 µg/ml
Spectrum	$Gram^+$ / $Gram^-$ / Mycoplasma

Neomycin

Cat. No.	4-10F00-H
Unit Size	100 ml
Presentation / ml	10000 µg
Suggested Working Conc.	50 µg/ml
Spectrum	$Gram^+$ / $Gram^-$

Puromycin 2HCl 10mg / ml

Cat. No.	4-17F00-BD/ -E
Unit Size	10 x 1ml / 20 ml
Presentation / ml	1–10 µg/ml
Spectrum	$Gram^+$ / $Gram^-$ (weakly)

Puromycin 2HCl (Powder)

Cat. No.	4-17P00-DB
Unit Size	100 mg
Presentation / ml	powder
Suggested Working Conc.	1–10 µg/ml
Spectrum	$Gram^+$ / $Gram^-$ (weakly)

Sera

Fetal Calf Sera (FCS)
Newborn Calf Sera (NCS)
Calf Sera (CS)
Newborn Horse Sera (NHS)
Horse Sera (HS)
Other Sera

Nudibranch
Nudibranchs are a large group of mollusks which are known for their often extraordinarily vibrant colours and bizarre appearances. There are over 3000 species of Nudibranchs, whose name comes from the latin word nudus, meaning naked, referring to the fact it sheds its shell after the larva stage. It is logical to think that their lack of shell would make them an easy target, however they are a relatively successful group. This is partially due to their bright colours warning off hungry predators, and partially because a number of types produce chemicals which make them taste terrible.

Colony of calcarious tubeworms

Sera

Special Serum Types

Standard Sera of different origins (South America, USDA approved, EU approved), mainly used for normal cell culture apllications.

Dialyzed Sera
BioConcept Ltd. offers dialyzed sera. Dialysis reduces the concentration of free low molecular weight components such as nucleotides, amino acids, hormones and ions. A dynamic filtration method is used to produce dialyzed sera.

Tetracycline Free Sera
BioConcept Ltd. offers tetracycline free sera. Sera are tested for the presence of chlortetracycline, oxytetracycline and tetracycline by a liquid chromatography electro spray ionisation tandem mass spectrometry method. The detection limit is < 0.05 mg/l.

Batch Testing
After batch testing BioConcept Ltd. can store prepaid reservations up to 24 months in our –20 °C storage rooms (free of charge) for customers who do not have enough storage facilities. Customers can call off their prepaid sera according to their demands.

Quality Control

Virus Testing
All calf sera are tested for adventitious viruses using cell culture techniques.
Test include:

– Bovine Viral Diarrhoea (BVD)
– Infectious Bovine Rhinotracheitis (IBR)
– Parainfluenza Type 3 (PI3)

All sera are tested for the absence of the indicated viruses by inoculation with GBK cells. The revelation is made by indirect immunofluorescence. Antibody testing: presence of specific antibodies is detected utilizing an elisa assay.

Mycoplasma
Each final product batch is tested for the absence of mycoplasma. All sera are tested for the absence of Mycoplasma utilizing a 21 days cell culture assay by the culture method.

Physical Parameters – Osmolality and pH
Osmolality is determined by the lowered freezing temperature. Osmometer and pH-meters are daily calibrated using standard solutions.

Endotoxins
All sera are tested to determine the levels of endotoxins. Two tests are performed: turbidimetric, a qualitative test, and chromo-kinetic, a quantitative test. The endotoxin reagent is standardized against the US reference endotoxin. The method is based on "the bacterial endotoxins test" USP.

Cell Culture Testing
Each final batch of serum is tested for its ability to support in vitro growth of specific cell lines. Therefore, in addition to verify that each batch of serum passes quality control specifications, three important performance criteria are evaluated, these are: Growth Promotion, Cloning Efficiency, Plating Efficiency. Following cell lines are used: L929, Hela, MRC-5 and Sp2/O-Ag14.

Heat Inactivated Sera
BioConcept Ltd. offers heat inactived sera. Heat inactivation is performed at 56 °C for 30 minutes. Heat inactivation destroys the complement which is needed especially in some immunology experiments. Heat inactivation can also inactivate viruses and bacterial contaminants such as mycoplasma.

Geographical BSE Risk Classification (GBR)

BioConcept Ltd. supplies a wide range of sera origins including sources from South America as well as from the European Union (EU) and from sources approved by United States Department of Agriculture (USDA).

The choice of FBS origin is determined by customer needs, import requirements and worldwide supply.

BioConcept Ltd. is the ideal partner for meeting different requirements, such as choosing the origin of serum, which provides optimal performance and results.

FBS is considered to be an animal by-product which is not intended for human consumption.

Biological safety is controlled by EU rules on animal by-products:

– Regulation EU 1069/2009 and regulation EU 142/2011
– Official disease status BSE, FMD, etc.

Individual countries are monitored for their animal health status by the World Organisation for Animal Health (OIE)

More information:
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003700.pdf

Fetal Calf Sera (FCS)

Cat. No.	Unit Size	Presentation	Origin
2-01F10-I	500 ml	Standard	South America EC approved
2-01F16-I	500 ml	Heat Inactivated	South America EC approved
2-01F17-I	500 ml	Dialyzed	South America EC approved
2-01F00-I	500 ml	Standard	Europe EC approved
2-01F26-I	500 ml	Heat Inactivated	Europe EC approved
2-01F28-I	500 ml	Tetracycline free	Europe EC approved
2-01F30-I	500 ml	Standard	Brasil
2-01F36-I	500 ml	Heat Inactivated	Brasil
2-01F40-I	500 ml	Standard	USA
2-01F46-I	500 ml	Heat Inactivated	USA

Newborn Calf Sera (NCS)

Cat. No.	Unit Size	Presentation	Origin
2-02F110-I	500 ml	Standard	Costa Rica

Calf Sera (CS)

Cat. No.	Unit Size	Presentation	Origin
2-03F00-I	500 ml	Standard	Europe

Horse Sera (HS)

Cat. No.	Unit Size	Presentation
2-05F00-I	500 ml	Standard
2-05F26-I	500 ml	Heat Inactivated

If you need sera not described please contact us. We can also supply you with other types of sera not mentioned in these lists.

Technical Information / Protocols

Guide to Supplementation of Media and Media handling

Standard Packaging and Flexibility

Protocols

Pacific sea nettle [*Chrysaora fuscescens*]
Sea nettles have a distinctive golden-brown bell with a reddish tint. Chrysaora fuscescens swim using jet propulsion by squeezing their bell and pushing water behind them, allowing them to swim against currents, although most of the time they prefer to simply float. Sometimes they pick up hitchhikers, including small fish and crabs, which hide inside the sea nettle's bell and may feed on it.

Glass shrimp or cleaner shrimp [*Periclimenes spec.*]

Technical Information

BioConcept Ltd.’s raw material and components are of the highest quality available. They are only purchased from approved and ISO-certified suppliers. All chemicals can be traced back to the lots used. During the manufacturing process the weights of the chemicals are controlled automatically by computerized balances. This guarantees a high degree of reproducibility of the formula. With our high quality water purification systems we can produce different levels of purified water, from standard purified water to WFI. This means we can offer ultra pure, apyrogenic water which is compliant to EP and USP standards.

The sizes of the batches produced start from 5 L, giving BioConcept Ltd. the flexibility to better serve its customers.

In BioConcept Ltd.’s quality control department, all liquid media are routinely tested for sterility, growth promotion and absence of cytotoxicity, the osmolality required and the correct pH level. Powdered media are reconstituted with cell culture grade water and checked as described for liquid media.

Guide to Supplementation of Media and Media handling

Unless noted otherwise, all 1 L liquid media are formulated without antibiotics, serum and L-Glutamine as Glutamine is the most unstable component in liquid cell culture media. 10 × concentrated media do not contain antibiotics, serum, L-Glutamine and sodium bicarbonate. Powder media contain L-Glutamine but no sodium bicarbonate. We recommend aseptically adding the required amount of these supplements prior to use. For the addition of the appropriate amounts of L-Glutamine and sodium bicarbonate, please refer to Table 1 below for the addition of antibiotics, please refer to Table 3 on **page 101.** Sodium bicarbonate can be added either as a solid before sterilization or as a 7.5% sterile solution after sterilization. Serum is usually added to the cell culture media to a final concentration of 1 to 25%.

Table 1: Guide to the addition of L-Glutamine (200 mM solution)

Medium (1 L)	L-Glutamine (200 mM/l) ml required	Final concentration of L-Glutamine (mg/L)
DMEM	20.0	584
DMEM/Ham F12 Standard	20.0	365
Ham's F-10	5.0	146
Ham's F-12	5.0	146
MEM Iscove's	20.0	584
Leibovitz L-15	10.3	300
M199 Earle's	3.4	100
M199 Hanks'	3.4	100
McCoy's 5A	7.5	219
MEM Alpha	10.0	292
MEM Earle's	10.0	292
MEM Hanks'	10.0	292
RPMI 1640	10.3	300
Waymouth's	12.0	350
William's E	10.0	292

Table 2: Guide to the addition of Sodium Bicarbonate (7.5% solution or powder)

Medium (1 L)	$NaHCO_3$ (7.5% solution) ml required	$NaHCO_3$ (solid) g required	Final concentration of $NaHCO_3$ (mg/L)
DMEM	49.3	3.70	3700
DMEM/Ham F12 Standard	32.5	2.438	2438
Ham's F-10	16.0	1.20	1200
Ham's F-12	15.7	1.176	1176
MEM Iscove's	40.4	3.024	3024
Leibovitz L-15	–	–	–
M199 Earle's	29.3	2.20	2200
M199 Hanks'	4.7	0.35	350
McCoy's 5A	29.3	2.20	2200
MEM Alpha	29.3	2.20	2200
MEM Earle's	29.3	2.20	2200
MEM Hanks'	4.7	0.35	350
RPMI 1640	26.7	2.00	2000
Waymouth's	29.9	2.24	2240
William's E	29.3	2.20	2200

Preparation of 1 L Single Strength Media from 10 × Concentrate

1. Aseptically measure out approximately 850 ml of sterile tissue culture grade water in a container of appropriate size.
2. Gently stir and aseptically add 100 ml of 10 × medium.
3. Now add the required amount of Sodium Bicarbonate.
4. Sera, antibiotics, L-Glutamine and other supplements may now be added to the solution.
5. Use sterile 1 N NaOH or 1 N HCl to adjust the pH of the solution to the desired value.
6. Use tissue grade water to adjust the final volume of the solution to 1 L.
7. The completed medium has a limited shelf life and should be refrigerated.

Preparation of 1 L Liquid Media From Powder Media

1. Place approximately 900 ml of cell culture water in a 1 L Erlenmeyer flask. While gently stirring, slowly add the powder. Do not heat.
2. Add solid Sodium Bicarbonate. Stir and adjust the solution with water to 1 L.
3. Control the pH and, if necessary, adjust with 1 N HCl to approximately 0.2 pH units below the value required after sterilization.
4. Use 0.2 µm filter to sterilize the medium.

Storage Conditions and Shelf Life of Media

Storage conditions and shelf life of media are indicated on the label.

Powder Media Use

Since it has been demonstrated that powdered media can be prepared by milling of the component chemicals, the use of powdered media has grown significantly. The use of a powdered medium allows the investigator uniformity that is not usually attainable from conventionally prepared liquid media. This is because a single batch of medium may be employed in long term or replicate experiments. Long term stability of powders and easy storage are two of the advantages of powdered media often cited. Autoclavable powdered media have not been employed routinely although the usefulness of such preparations is readily apparent. Succinic acid and sodium succinate are utilized to maintain the pH of 4.0–4.5 during autoclaving.

Media with Stable Glutamine

In liquid solutions, L-Glutamine is not stable at temperatures of 4 °C or higher. The molecule dissociates and creates toxic ammonium as a waste product of that reaction. This restricts the stability of cell culture media and as a consequence, stock solutions of L-Glutamine or solutions containing Glutamine have to be stored frozen. Additionally, cell culture media have to be changed frequently to ensure the availability of the amino acid.

Dipeptides containing Glutamine are extremely well suited as a replacement of L-Glutamine. They are stable over long periods of time, even if stored at room temperature. Cells may recover L-Glutamine via cleavage of the peptide bond using cellular peptidases. BioConcept Ltd. offers several media with stable Glutamine in the form of L-Ala-L-Gln (Cat. No. 5-10K50-H). The amount of available L-Glutamine corresponds to that of L-Glutamine normally present in the equivalent medium after the addition of the appropriate amount of L-Glutamine.

Media with HEPES

If you have a sensitive cell culture system, we recommend that you use HEPES buffered media. Such nutrient solutions are prepared in accordance with the original formulation and include Sodium Bicarbonate. However, to maintain an appropriate osmolality, the concentration of Sodium Chloride is decreased. HEPES increases the buffering capacity and the stability of the pH in the range of 7.2 to 7.6.
This allows the media to better resist fluctuations in the pH resulting from changes of cellular metabolism especially in rapidly growing cultures with a strong acidulation. BioConcept Ltd. offers several media with HEPES (see section Special Media).

Media without Phenol Red

Phenol Red is not a natural substance. Nevertheless, it is added as an indicator of the pH to cell culture media. However, the presence of this dye is not always desired. Especially in the cultivation of primary cells, media with Phenol Red may produce artifacts. Additionally, Phenol Red may interfere with chromogenic bioassays. BioConcept Ltd. offers several media without Phenol Red, if you cannot find your media, please contact BioConcept Ltd. using the information at the back of this catalogue.

Deficient Media

Deficient MEM media are suited for radioactive incorporation studies to investigate metabolic aspects of cellular physiology. BioConcept Ltd. offers several deficient MEM-media. We can produce any deficient media, including media used in NMR labelling experiments

Serum-Free and Protein-Free Media

Normally, serum has to be added to cell culture media. It has multiple functions, for example, it may serve as a source of nutrients, enzymes, hormones and growth factors; it may also serve as a pH-buffer or may bind toxic components. However, serum has quite a lot of disadvantages; it is expensive, shows lot-to-lot-variability and increases the complexity of the down-stream processing of the desired biological material. BioConcept Ltd. offers several serum-free and protein-free media.

Preparation of Customer-Designed Media

We make specialized modification to standard cell culture media as well as nutrient mixtures that are adapted to cell requirements. In our modern manufacturing plant we produce under strictly controlled conditions to create formulations. Our employees have many years of experience in the production of cell culture media and we have extensive in-process and end-controls to ensure that your product is of the utmost quality.

If you want to order your special media, please e-mail us your formulation at info@bioconcept.ch. If you have additional questions on your special media please do not hesitate to contact us!

If you are interested, BioConcept Ltd. would like to show you its production plant in Allschwil, Switzerland. If you would like to know how we manufacture your cell culture media, please contact your local BioConcept Ltd. representative or directly call + 41 (0)61 486 80 80.

Serum Handling Tips

At BioConcept Ltd., our goal is to provide maximum value with our products to all of our customers. To help ensure that you get satisfactory performance from your order of BioConcept Ltd. serum, BioConcept Ltd. recommends that you adhere to the following handling guidelines.

BioConcept Ltd. serum should be inspected on arrival and stored at –20 °C. Should the serum become partially thawed, we recommend that you leave it to thaw completely at room temperature and then carefully mix, avoiding frothing. The serum may be refrozen and stored at –20 °C before use. In our experience, this will not decrease the growth promoting properties of the serum, and will help prevent precipitates and complexes from forming in the serum. Avoid glass-to-glass and glass-to-metal contact. Most glass breakage is caused by bruising resulting from this type of contact. Avoid rapid temperature changes. Never transfer a glass bottle directly from a freezer to a water bath. Conversely, avoid transferring glass bottles directly from a water bath to a freezer. Always keep some space between serum bottles during storage.

Thawing Serum: When serum has been stored at –20 °C, it should be allowed to equilibrate in a refrigerator at 2–8 °C (preferably overnight). Placing a piece of cloth

under the bottles during equilibration helps reduce glass breakage. Allow thawing to complete at room temperature. Thawing serum at high temperatures is not recommended. Periodic agitation during thawing is helpful. Turbidity or flocculent material may appear upon thawing or storage. This is caused by lipoproteins that may precipitate. This will not adversely affect the performance of the product. Complement is inherent in sera and can be inactivated by raising the temperature to 56 °C (heat inactivation).

Heat Inactivation: BioConcept Ltd. offers FCS in a heat inactivated form upon request. However, it is a rather simple procedure to inactivate serum. Essentially, all that is needed to be done is to incubate the serum at 56 °C in a water bath for 30 minutes. The serum must be gently swirled every 10 minutes during this incubation. Please be advised that heat inactivation may cause serum gelation. Sera with higher protein concentrations will gel more easily. Also, heat inactivation at higher temperatures or for longer periods of time may promote gelation.

Using Antibiotic/Antimycotic solutions

The following table is a general guide for usage of antibiotics in cell culture media containing serum. Serum-free media require lower concentrations and should be determined empirically. Stability of these antibiotics in cell culture media is at least 3 days at 37 °C.

Table 3: Guide for the addition of antibiotics

Cat. No.	Product Description	Presentation/ml	Suggested Working Concentration	Spectrum
4-01F00-H	Penicillin/ Streptomycin	10 000 IU/ 10 000 µg	1:100	Gram$^+$, Gram^2
4-02F00-H	Penicillin/ Streptomycin/ Fungizone®	10 000 IU/ 10 000 µg/25 µg	1:100	Bacteria, Fungi, Yeasts
4-03F00-H	Penicillin/ Streptomycin/ Fungizone®	10 000 IU/ 10 000 µg/250 µg	1:100	Bacteria, Fungi, Yeasts
4-05F00-H	Amphotericin B/ Fungizone®	250 µg	1:100	Fungi, Yeasts
4-07F00-H	Gentamicin	5 000 µg	1:100	Gram$^+$, Gram^2, Mycoplasma
4-10F00-H	Neomycin	10 000 µg	1:200	Gram$^+$/Gram^2
4-15F01-H	G418/Geneticin®			
Used as a selective agent in mammalian cells at concentrations ranging from 400 to 1 000 µg/ml. G418 blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic cells. Resistance to G418 is conferred by the Neomycin resistance gene (neo) from Tn5 encoding an aminoglycoside 3'-phosphotransferase, APH 3' II.				
4-15P01-GA	G418/Geneticin® crystalline			
Used as a selective agent in mammalian cells at concentrations ranging from 400 to 1 000 µg/ml. G418 blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic cells. Resistance to G418 is conferred by the Neomycin resistance gene (neo) from Tn5 encoding an aminoglycoside 3'-phosphotransferase, APH 3' II.				
4-21F01-E	Hygromycin B			
Used as a selective agent in molecular genetics experiments on a wide variety of eukaryotic and prokaryotic species. The hph gene confers hygromycin-resistance to cells expressing it. Mammalian cells are sensitive to Hygromycin B concentrations of 50–200 µg/ml, and bacteria to 50–100 µg/ml.				

Biological Buffers and pH Control

All media used in tissue culture have a synthetic mixture of inorganic salts known as a 'physiological' or balanced salt solution. The basic functions of this salt solution in the medium are to maintain proper pH, maintain ideal osmotic pressure, and provide a source of energy. The growth of animal cells in a nutritionally complete tissue culture medium is usually optimal when the medium is buffered at a pH in the range of 7.2–7.4. To function most effectively, the pKa of the chosen buffer should be as close as possible to the required pH.

The most commonly used buffer in tissue culture media is sodium bicarbonate. However, this buffer has two important disadvantages:

1. The pKa of sodium bicarbonate is 6.3 at 37 ˚C which results in suboptimal buffering throughout the physiological pH range, and
2. Carbon dioxide is released in the atmosphere which results in an increase in alkalinity, and the number of hydroxyl ions produced increases according to the amount of sodium bicarbonate added to the medium. It is possible to control this by artificially supplying carbon dioxide to the atmosphere and preventing the gas from leaving the liquid, thereby reducing the hydroxyl ion concentration in solution.

Balanced salt solutions can be divided into two types: those intended to equilibrate with air in a closed system at a low concentration of sodium bicarbonate (Hanks' Balanced Salt Solution) and those intended to equilibrate with a gaseous phase containing approximately 5% CO_2 at a higher concentration of sodium bicarbonate (Earle's Balanced Salt Solution Cat. No. 3-01F00-I). Earle's Balanced Salt Solution is a much better solution because it contains a greater amount of sodium bicarbonate, but it is more difficult to use since it requires a special gaseous mixture of 5% CO_2 with 95% air to be provided by the culture medium. If this procedure is not carried out, the pH increases rapidly at normal incubation temperatures. The purple color of the medium indicates that the pH has risen, and cell growth is inhibited. An alternative method is to use a medium which produces sufficient buffering capacity but does not require 5% CO_2.

In some cases, this can be achieved by using a medium containing Earle's salts but having the concentration of sodium bicarbonate reduced to 0.85 g/liter. An entirely different approach was devised by Leibovitz (1963). He utilized the buffering capacity of free base amino acids, omitted sodium bi-carbonate, substituted galactose for glucose and added pyruvate. The pH of his L-15 medium is approximately 7.8 which is higher than that of most other media. Since there is no production of loss of CO_2, the pH will not rise further. This medium makes possible the growth of cells in open culture vessels without regard to the CO_2 content of the atmosphere.

Attempts have been made in recent years to find the most suitable buffer. The most commonly utilized alternative to bi-carbonate is HEPES buffer which was first described by Good, et al. (1966). It acts as a zwitterion and has proved superior to conventional buffers in comparative biological such as with cell-free preparations. It has many properties which make it ideal as a buffer to tissue culture media, principally in that it does not require an enriched atmosphere to maintain the correct pH. HEPES does not bind divalent cat ions and is soluble to the extent of 2.25 M at 0˚C. **Note:** since the DpKa/°C of 20.014, the pH reading recorded in a HEPES buffered medium will vary inversely with the temperature of the medium.

Expected pH levels at various temperatures

°C	pH	°C	pH
5	7.58	23	7.47
15	7.56	24	7.46
16	7.55	25	7.44
17	7.54	26	7.43
18	7.53	27	7.42
19	7.52	28	7.41
20	7.50	29	7.40
21	7.44	30	7.38
22	7.48	37	7.30

HEPES (Cat. No. 5-31F00-H) is satisfactory as a buffer in tissue culture media for the growth of many different types of cells and viruses. It may exhibit toxicity at concentrations greater than 40 mM. Studies have indicated that 20 mM HEPES is the most satisfactory concentration for the buffer when both Hanks' and Earle's solutions are used. CO_2 incubators should not be used with media buffered solely with HEPES. BioConcept Ltd. HEPES buffered liquid media are produced with a pH of 7.2–7.4 at 37˚C. Powdered media are prepared so that a 10x solution will have a pH of 7.2–7.4 at 37˚C without further adjustment.

Sodium bicarbonate should also be added as a nutritional requirement. It is recommended that the sodium bicarbonate concentration should not exceed 10 mM when the HEPES concentration is 20 mM. All single-strength (1 L) liquid media contain either sodium bicarbonate or HEPES buffer or both. 10× concentrated (10 L3) liquid media do not contain buffer and powdered media are either buffer free or contain HEPES buffer only. Whenever sodium bicarbonate is used to buffer tissue culture media at a concentration of greater than 1.0 g/L, a CO_2 enriched atmosphere is required, which can be created through using a CO_2 incubator.

Mammalian cells can survive over a wide pH range 6.6–7.8, but as a rule, the optimal growth of cells is obtained at pH 7.2–7.4. It is undesirable to allow the pH to deviate outside the limits of pH 6.8–7.6. It should be remembered that no buffer is capable of holding the pH constant in a system in which acids or bases are being produced. Buffers only slow the rate of pH change. Cells in culture produce acidic products which act to lower the pH of the medium. Most of the media utilize phosphates and the bicarbonate system to buffer the media. The bicarbonate ion can be converted to gaseous carbon dioxide and lost from the medium resulting in a rise in the pH. Carbon dioxide can be maintained by supplying a special gas phase or by sealing the vessel tightly so that the CO_2 produced by metabolic processes is retained in the vessel and re-absorbed by the medium. Efforts to eliminate bicarbonate as a buffer system have been reported in medium SR1-8 and in medium L-15. Both media contain a phosphate buffer and increased amounts of amino acids to accomplish the buffering required.

A number of organic compounds have been described that have buffering capacity in the required range. HEPES will have a pronounced effect on the final pH. It is necessary to measure the pH at the temperature of use to determine the final pH due to the contribution by other buffers. HEPES buffer may be sterilized by steam and adjusted to desired pH with sodium hydroxide. Concentrations of 10–25 mM have been employed with no apparent toxicity. When utilizing HEPES, BioConcept Ltd.

chooses a 15–25 mM concentration in the preparation of media unless it is otherwise specified. A mixture of acid form and base form HEPES is used in combination in most of BioConcept Ltd.'s media at this has been found to produce a more stable buffering system. Sodium chloride is reduced to keep the osmolality of the media containing both bicarbonate and HEPES in the range on non-HEPES containing media.

Aseptic Techniques

70% of all problems are due to a lack of sound aseptic technique. Microorganisms which can result in contamination exist everywhere, from the surfaces of objects to the surrounding air. A conscious effort must be made to keep them out of the sterile environment. Because cell culture techniques often entail several steps which can lead to contamination, cell culture media are often supplemented with antibiotics. Purchasing cell culture media from premium vendors such as BioConcept Ltd. where quality assurance measures are rigorous will also greatly reduce the incidence of contamination. Nevertheless, this will not eliminate cell culture contamination resulting from poor sterile technique or antibiotic resistant mutants. Autoclaving will render pipettes, glassware and solutions sterile. Nutrient media cannot be autoclaved. The compounds in nutrient media are destroyed by the heat generated during autoclaving. Media must therefore be sterilized by sterile filtration through filters small enough in pore size to hold back bacteria and mycoplasma.

Guidelines for Sterile Techniques

1. Wipe your work area clean with 70% ethanol which has been sterile filtered prior to use. Filtration is required as 70% ethanol can be a good preservative for Fungi spores (Cat. No. 10-03F12-I). It may help to rinse your hands with ethanol as well.
2. Keep sterile flasks, bottles, and petri-dishes covered until the instant of use. Return any covers as soon as you are finished.
3. Sterile pipettes should NEVER be taken from the cylinder or wrapping until they are ready to be used. Keep your pipettes at your work area. Sterile pipettes DO NOT have to be flamed. Pipetting your cells with a hot pipette will kill them.
4. When removing the cap from a bottle, flask, etc., keep it face down, grasping it with the little finger of your right hand. DO NOT place caps on the lab bench. Flame the lips of flasks, bottles and other culture ware before and after introduction of a pipette. DO NOT hold the opening straight into the air. If possible, tilt the container such that any falling microorganisms will land in the lip where they will be flamed upon closure.
5. Avoid talking, singing or whistling while performing sterile procedures as these generate aerosol contaminants.
6. NEVER PIPETTE BY MOUTH. Use a pi-pump or other device. Even though your pipettes are plugged, mycoplasma in your mouth can still pass through and become one of the largest problems in cell culture contamination.
7. DO NOT draw from a bottle more than once with the same pipette. Use a sterile pipette each and every time – especially when pipetting media!
8. Techniques should be performed as rapidly as possible to minimize contamination. It is possible that you may find yourself performing a procedure not addressed by these sterile technique guidelines. Therefore, you must constantly be aware that microorganisms are everywhere and take proper steps to keep them out of your cultures. When first developing your aseptic technique, you must always think of sterility. Eventually, it will become second nature to you. Mastering good aseptic technique will save minimization of biohazard risk when infectious organisms or dangerous chemicals are being used.

Adaptation of Cells to Serum

The majority of established cell types currently cultured in media supplemented with FCS can be adapted to grow with BioConcept Ltd. Calf, Donor Calf or New-born Bovine Serum. Complete adaptation may take between three to four subcultures and should be carried out using a 10% serum concentration. Cells growing in smaller serum concentrations should be brought back to 10% serum before adaptation is attempted. The following method is a basic guide to the procedure:

1. Two days prior to sub culturing, nourish the cell line with defined medium containing 5% FCS and 5% alternative serum (known as "adaptation serum").
2. 48 hours afterward, trypsinize the cells and split them using "adaptation medium".
3. Nourish the cells with "adaptation medium" until confluent.
4. Repeat steps 2 and 3 for 1–2 subcultures.
5. Split the confluent cells using media containing 10 % alternative serum.
   Note: Most heteroploid lines can be grown on a variety of sera without affecting the growth rate. Diploid lines are more fastidious but can be adapted. Primary cell cultures should be initiated on newborn calf serum wherever possible.

Eye Examination of Cell Cultures

Before commencing the general health of any culture should be evaluated. This can be done quickly and quantitatively by making the following observations.

1. Check the pH of the culture by examining the color. As a culture becomes more acidic, the indicator (phenol red) shifts from red to yellow. In contrast, as a culture becomes more alkaline, its color shifts from red to purple. In general, cells can tolerate slight acidity better than slight basicity. Any shift above pH 7.6 is particularly detrimental. Until you become familiar with the color of the indicator at various pH levels, use color standards for comparison.
2. Cell attachment of monolayer cultures should be to the well and spread out evenly. If cells are floating in the culture, determine if they are in a state of division or if they are dying. A dying cell will have an irregular morphology (alternatively, you can test for cell viability using Trypan Blue from BioConcept Ltd. (Cat. No. 5-72F00-H).
3. The “percent confluency” or growth rate of a culture can be estimated by following it toward the development of a full cell sheet (i. e. a “confluent culture”). By comparing the amount of space covered by cells with the unoccupied spaces you can estimate the percent confluency.
4. The cell shape is an important guide. Round cells in an uncrowded culture are not a good indicator unless these happen to be dividing cells. Look for doublets or dividing cells. Get to know the effect of crowding on cell shape.
5. Look for “giant cells”. The number of “giant cells” will increase as a culture ages or declines in “well-being”. The frequency of “giant cells” should be relatively low and constant under uniform culture conditions.
6. One of the most valuable early indicators in assessing the success of a “culture split” is the rate at which the cells in the newly established cultures attach and spread out. Attachment within an hour or two suggests that the cells have not been traumatized and that the in vitro environment is not grossly abnormal. Longer attachment times are suggestive of problems. Nevertheless, good cultures may result even if attachment does not occur for four hours.
7. Learn to recognize the range of cell shapes and growth patterns exhibited by each cell line. For example, many transformed cells will “pile up” due to a lack of contact inhibition. This effect becomes more pronounced as the culture becomes overcrowded.

Mycoplasma Contamination

Mycoplasma are prokaryotic cellular organisms and are the smallest cells capable of autonomous growth. They lack a rigid cell wall and are pleiomorphic. Additionally, they are sensitive to oxygen tension and osmotic shock. Many of the smaller mycoplasma can pass through bacterial filters. The cell size ranges from 0.15 to 1.0 µm. Mycoplasma can cause economically important infections of the respiratory tract, mammary glands, genital tracts or synovia of cattle, sheep, goats, cats, mice, rats, pigs and poultry. Some effects of mycoplasma infection on cell culture have been reviewed (McGarrity, et al., 1984). These infections DO NOT always result in microscopic alterations of cells or media. Many infections grow slowly and do not de-story host cells but can still alter the metabolism of the culture in subtle ways. The exact manner in which contamination of cell cultures occurs may be impossible to ascertain in any given instance. Studies by Barile and others (Barile, et al., 1978) have demonstrated that mycoplasma infection or contamination of cultures is the result of a number of factors, involving the investigator, the type of cell culture used and the media used in handling the cells. McGarrity (1976) concluded that mycoplasma-infected cultures are the most common source of further contamination. He listed recommendations for prevention and control of mycoplasma infection. These can be summarized as the following combination of practices:

– The use of primary cultures rather than propagated cell types.
– The use of heat-inactivated serum.
– The avoidance of direct mouth pipetting.
– The strict enforcement of good aseptic techniques.
– The use of antibiotic free media.
– The use of protective clothing by technicians.
– The use of premium cell biology vendors such as BioConcept Ltd. where maximum QC measures are employed.
– The use of prophylactic tools.
– The use of high concentration of gentamicin can reduce Mycoplasma contamination.

Subculturing Protocol Anchorage-Dependent Cells (Short Method)

1. Decant supernatant fluid from the culture plate into a waste collection jar, taking care to use sterile technique.
2. Add 3 ml of cold trypsin (Cat. No. 5-51F00-H) to the culture flask.
3. Incubate for 30 seconds (or longer if necessary). Examine at low magnification. When it appears that some of the cells have rounded up, but have yet to detach, decant the trypsin into a waste container. Continue to incubate the flask until virtually all cells have rounded up and can be readily dislodged.
4. Using a 10 ml pipette, add 10 ml of fresh MEM (Cat. No. 1-31F01-I) to the T-flask. Dispensing the MEM as a strong stream will aid in dislodging the cells.
   Note: 10 ml is sufficient for a 1:2 split. Next, using the same pipette, draw up the cell suspension and quickly dispense a 5 ml aliquot into two 25 cm^2 T-flasks (one new, one old).
5. Incubate and monitor the flasks periodically beginning 30–45 minutes after inoculation. Rapid attachment (usually within 1 hour) indicates that the split has been successful.
6. Continue to monitor the culture's progress.

Always employ sterile techniques throughout. The above volumes correspond to a 25 cm^2 T-flask.

Haemocytometer Counting and Cell Viability

Accurate enumeration of cell density is an important aspect of cell culture. Cell enumeration with a haemocytometer is the most widely used method, and it continues to be used in most cell culture laboratories including those equipped with electronic cell counters. The following review information should help in proceeding through the cell counting protocol. Cell populations are usually expressed as the number of cells/ml or as the number of cells/culture.

When viewed with a compound microscope with a 10× ocular and a 10× objective (total magnification 100×), one large square of the haemocytometer of 1×1 mm will fill the field. When using a Neubauer haemocytometer, each of the four large corner squares and the large center square are counted. When the cell count is low, 10 cells/square, all nine squares should be counted. Each large square measures 1×1 mm and is 0.1 mm deep. Hence, each large square has volume of 0.1 mm^3 or 0.0001 ml (10–4 ml). The final calculation takes into account:

1. How you wish to express your count (cells/ml or cells/flask).
2. The dilution (amount of saline, dye or media in ml which your cells have been suspended).
3. The number of squares counted. Hence, the number of cells/ml can be calculated as:
   – Total Number of Cells Counted
   – Total Number of Squares Counted
   – Trypan blue stains dead cells and therefore allows the calculation of live cells only.

Proceed to make a 1:2 split of the culture. In order to determine the number of cells which were harvested from the flask, the culture must be centrifuged, the supernatant decanted and the pellet resuspended in 1 ml MEM. Aseptically remove 0.1 ml of cell suspension and mix while adding to a dilution tube containing 0.8 ml BSS and 0.1 ml trypan blue (0.4%). Sterility need not be maintained from this point forward. After 3–4 minutes, remove a few drops of the cell suspension with a Pasteur pipette and load both haemocytometer chambers after putting the coverslip in place. Count the total number of cells and the number of trypan blue stained (dead) cells in each of the four corner squares and the central square. Proceed to count as follows:

1. For total cell number in dilution tube (1 ml), it is calculated as follows: Total Cell Count 3×10^4 divided by 5 (number of squares counted)
2. For non-viable cell number in dilution tube (1 ml), it is calculated as follows: Total Stained Cell Number 3×10^4 divided by 5
3. For total number of cells harvested from the original flask, it is calculated as follows: Total Cell Count 3×10 (dilution factor*)×10^4 divided by 5
   *A dilution or multiplication factor of 10 is used here because the aliquot removed for counting (0.1 ml) is one tenth of the total cell suspension in the centrifuge tube.
4. For number of cells/new culture after inoculation of the flask with 0.4 ml of cell suspension and addition of 5.6 ml MEM, it is calculated as follows: Total Cell Count 3×4 times 10^4 divided by 5

Cell Freezing

At BioConcept Ltd., we store our cell lines in cryotubes designated "Seed Stock" and they are frozen as soon as possible after packaging. The freezing of cells is used to preserve seed stocks of any given culture, to guard against "genetic wandering" and contamination, and for long term storage of a culture not in regular use.

BioConcept Ltd. recommends that after obtaining a fresh stock of cells from primary sources, a proportion of the culture material be preserved and frozen as early as possible. Ideally, this should be done within 1 or 2 passages after obtaining the cell culture. A "seed" stock of culture material may be withdrawn at intervals, thereby ensuring a constant, identical source of cells for many years to come. From the seed stock, further aliquots are taken, frozen and designated as "working stock." This "working stock" has sufficient ampoules for approximately one year. When the "working stock" has been depleted, another ampoule of seed stock is thawed and grown up to provide a new supply of working stock.

Using this procedure, almost unlimited supplies of genetically similar material can be ensured. We do not recommend handling cells for more than 10 passages or 10 weeks in the laboratory. This ensures that changes will not occur in the cell line. It is essential that fresh back-up stocks always be available in deep-freeze. When freezing cells, the following recommendations ensures high cell viability:

1. Check that cells are rapidly growing and in a phase of exponential growth before freezing. If working with finite, fibroblast cultures, they should NOT have been "confluent" for more than 24 hours. If working with a continuous cell line, harvest at 70–80% confluency. Suspension cells should be spun and harvested while in exponential growth.
2. Trypsinize the monolayer as quickly as possible, using cold trypsin to minimize trypsin carry over. Use only minimal amounts of trypsin, ensuring that most is removed from the monolayer. This may be achieved by simply pouring off the trypsin or by centrifuging the cells and resuspending them in fresh medium.
3. Freeze the cells at a concentration between 2 and 5×10^6 cells/ml. For finite cultures, it is recommended that the entire contents of a flask be harvested, spun and the cell pellet resuspended in sufficient medium to produce one ampoule of

material (i.e. enough to generate one flask worth of cells at passage number 15). If two ampoules are produced from one flask, then this must be accounted for in the passage number, which in this case, a 1:2 split has been accomplished, so the passage number would be 16.

4. For established cultures, the yield is often large enough for several ampoules to be produced from one parent flask. In this case, the passage number should be increased by 1 to indicate that a passage or the equivalent of a subcultivation has occurred.
5. Keep the monolayer suspension COOL to minimize damage from the cryoprotective reagent. Since suspension cells do not respond well to low temperatures, they should NOT be cooled. Place the ampoule in a controlled rate freezer that is set to cool at a rate of 1 °C per minute. Allow the cells to freeze for approximately four hours before transferring them to a permanent position in the nitrogen freezer.
6. Frozen cells may be kept for short periods (4 – 10 weeks) at – 20 °C, but long term storage should always be in the vapor phase of liquid nitrogen or an equivalent temperature.

Frozen Culture Recovery

Thaw the frozen ampoules by gently swirling the vial in the 37 °C water bath until there is just a small bit of ice left in the vial. Transfer the vial into a laminar flow hood, wipe the outside of the vial with 70 % ethanol or isopropanol to decontaminate it prior to proceeding with the thawing procedures. Remove the cells with a narrow pipette or syringe and transfer them drop wise over 1 – 2 minutes to a centrifuge tube or a culture flask filled up with pre-warmed complete medium appropriate for your cell line. A 1:10 dilution should be made. Centrifuge the cell suspension at approximately 200 × g for 5 – 10 minutes. The actual centrifugation speed and duration varies depending on the cell type. Gently resuspend the cell pellet in fresh growth medium and transfer them into the appropriate cell culture vessel. If the cryoprotective reagent is NOT removed by centrifugation it is recommended to feed the cell culture after 24 h incubation with the growth medium.

Subculturing Protocol Established, Anchorage-Dependent Cell Lines (Long Method)

1. Aseptically decant growth medium from the flask and replace with EBSS without calcium and magnesium (Cat. No. 3-01F29-I) as follows:
   a. 25 cm² flask approximately 5 ml
   b. 75 cm² flask approximately 10 ml
   c. 120 cm² flask approximately 25 ml
2. Carefully wash the monolayer with a salt solution to remove any excess serum.
3. Aseptically decant the salt solution and replace with an equal amount of 0.05 % trypsin/0.02 % EDTA solution (Cat. No. 5-51F00-H).
4. Pass the trypsin/EDTA solution over the monolayer several times by gently racking the flasks and decant. DO NOT allow the trypsin/EDTA to be in contact with the monolayer longer than 30 seconds.
5. Lay the flask flat and incubate at room temperature until the cells detach. Flasks which have been trypsinized should be inspected regularly to prevent the cells from remaining in the trypsin/EDTA longer than is needed.
6. Wash the cells off the base of the flask with 10 ml of the required growth medium, taking special care with cells which may still be adhering to the sides and shoulders.
7. Aspirate the cell suspension carefully to break down any cell aggregates. Avoid excessive frothing.
8. Proceed as per experimental protocol or split cells as required for stock.
9. At all times, be aware of the possibility of cross-contamination. NEVER mix caps or reuse a pipette.

References

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4. Barile, M. F., Hopps, H. E. and Grabowski, M. (1978) Incidence and source of mycoplasma contamination: a brief review, In: “Mycoplasma Infection of Cell Cultures” (McGarrity, G.J., Murphy, D.G., and Nichols, W.W., Eds.), pp. 35 – 46. Plenum Press, New York.
5. McGarrity, G. J. (1976) Spread and control of mycoplasmal infection of cell cultures. In Vitro 12:643 – 648; 1976

Standard Packaging and Flexibility

BioConcept Ltd. has a large variety of containers available to our customers. When packaging liquid media we fill 650 ml bottles for the standard 500 ml order and for 100 ml orders we use the smaller 125 ml bottles. Sartorius Flexboy® and Flexsafe® bags are used for various volumes from 1 up to 500 liters.

We handle all kinds of containers when packaging powder media, this includes securibox's for sizes up to approximately 1 kg. Plastic containers are used for orders up to 25 kg and for greater quantities plastic drums are adopted. The price of packaging is included with the product, so there are normally no additional costs.

At BioConcept Ltd. we are proud of our ability to listen to our customers and offer flexibility in meeting their needs. Therefore, customized packaging is also available. If you have specific container requirements please specify when placing your order.

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1 Sterile Bags

Sterile Bags are a single-use alternative to glass, metal and plastic containers. They are an effective means to transport media, are shatter-proof and are easy to install for our customers. Furthermore, the bags have been sterilized using gamma radiation to ensure the media does not become contaminated. Flexboy® and Flexsafe® bags fully compliant with ISO11137, the highest sterility assurance level.

2 Transfer Sets (also customized)

Delivery of medium from media-bags to e.g. bioreactors requires various tubings and transfer-sets. BioConcept Ltd. offers solutions according to customers specifications.

3 Plastic Drums

The drum material is made from a high quality plastic and extensive tests are carried out to ensures the containers are secure. The quality standards are also in accordance to the UN's Recommendations for the Transport of Dangerous Goods, to ensure security. The drums are reusable and also recyclable, to reduce waste and an environmental impact. At BioConcept Ltd. large quantities of powders are shipped using such containers.

4 Flasks

BioConcept Ltd.'s flasks are manufactured by the Thermo Scientific company. The material used to make the flasks are strong, leak proof and ideal for cell culture media. The shape of the flasks also make for easy storage in bulk and as individual flasks. A lot of BioConcept Ltd.'s smaller volume orders of cell culture media are shipped in such containers. The flasks are sterilized before entering the clean room and are recycable.

5 SecuriBoxes™

For smaller orders of our powder medium, or on the customer's request, the product is packed into Securiboxes. The containers are strong and are the suitable dimension for easy storage and use. The containers are created by the Joma company in Austria in an ISO 7 production facility. The boxes are rigorously tested according to pharmaceutical industry guidelines for ISO 15378:2207.

6 Transport Container

The large steel containers seen in this photograph hold 500 liter media bags. The steel container protect the bags from any potential puncturing or damage during storage and delivery. The bags placed in the steel container are filled in the sterile room and then quickly transferred to the storage rooms, where they are kept at the temperature required for the media.

If you have any questions then please contact us by e-mail: info@bioconcept.ch

Protocols

Reconstitution of MAM-PF®7e Powder (Animal Component Free) Results in MAM-PF77 liquid

Required Supplements

1. L-Gln (200 mM)
2. Lipid Mix (1 000 ×) sterile, (Cat. No. 5-77F02)
3. Supplement 1 for 10-02P74 (Cat. No. 5-09Z08)
4. Supplement 2 for 10-02P74 (Cat. No. 5-09Z12)
5. MAM-PF77 Suppl. 1 (Cat. No. 5-03Z78)
6. MAM-PF77 Suppl. 2 (Cat. No. 5-03Z79)
7. MAM-PF77 Suppl. 3 (Cat. No. 5-03Z80)
8. $NaHCO_3$ (1.945 g/L)

Reconstitution of MAM-PF®7e Powder (Cat.No. 10-02P74) Results in 1 L liquid (10-02S80)

1. Dissolve 23.80 g powder (Cat. No. 10-02P74 in 900 ml bidistilled water or equivalently purified H_2O)
2. Add L-Glutamine (200 mM) to required final concentration (recommended 0.6 – 8 mM)
3. Add 1 ml Lipid Mixture (1 000 ×), (Cat. No. 5-77F02)
4. Add 1.85 ml Supplement 1 for 10-02P74 (Cat. No. 5-09Z08)
5. Add 20 ml Supplement 2 for 10-02P74 (Cat. No. 5-09Z12)
6. Add 2 ml MAM-PF77 Supplement 1 (Cat. No. 5-03Z78)
7. Add 1ml MAM-PF77 Supplement 2 (Cat. No. 5-03Z79)
8. Add 1 ml MAM-PF77 Supplement 3 (Cat. No. 5-03Z80)
9. Add 1.945 g/L $NaHCO_2$
10. Adjust pH to 7.10 – 7.20
11. Add bidistilled water (or equivalently purified H_2O) to final volume (1 L medium)
12. Filtration: 0.2 µm filter

Express Media for Hybridoma Cells

Product Handling

The optimal culture conditions are 37 °C with 5 % CO_2. When cultured in flasks, the lid should be slightly opened for gas exchange.

Adaptation of cells to serum-free conditions

In most cases is a direct cultivation of hybridoma cells in ISF-1 possible. Nonetheless, the following protocol for a stepwise adaptation has been proven successful. The successful adaption also depends on the character of the used hypridoma cell line. Therefore, it is highly recommended that a control culture is continued to be kept in its original medium until the adaptation to ISF-1 has been successful. The cell culture should be in its logarithmic growth phase with a minimum rate of living cells of 90 %.

Direct adaptation

1. Subculture of cells from serum containing media into 37 °C pre-warmed ISF-1. Cell density should correspond to the one used in the original culture.
2. Incubation of cells at 37 °C with 5 % CO_2.
3. Passaging of cells under strict control of its growth curve and viability for a minimum of 4 – 8 passages.
4. If growth and viability is significantly reduced during the 4 – 8 passages, the stepwise adaptation protocol should be used.

Stepwise adaptation

1. Seeding of cells with double the density compared to its normal inoculum into a 75:25 (v/v) mixture of serum containing to serum-free medium.
2. Subculture of cells when density reaches 10^6 viable cells/ml into a mixture of 50:50 (v/v) serum containing to serum-free medium.
3. Subculture of cells when density reaches 10^6 viable cells/ml into a mixture of 25:75 (v/v) serum containing to serum-free medium.
4. Subculture of cells when density reaches 10^6 viable cells/ml into ISF-1.

SERUM FREE CULTURE in SF-4 Baculo Express Medium

SF-4 Baculo express medium is a proprietary formulation which has successfully been used to grow various *Spodoptera frugiperda* (SF9, SF21) , BTI-TN-5B1-4 (High Five™) and *Drosophila melanogaster* (D.Mel-2) cells.

1. Guidelines for adaptation to SF-4

Special regimens are required to adapt insect cells from serum-containing to serum free SF-4 media: direct and gradual replacement (weaning). It is critical that the cells are in exponential growth before medium replacement and that the minimum cell density is at least 2×10^5 cells/ml medium.

a. Direct medium replacement

1. Grow the cells to about 60 % confluence (anchored cultures) or a density of 2×10^6 cells/ml (suspension cultures) in serum containing media.
2. Harvest the cells, dilute to 5×10^5 cell/ml serum-free SF-4 medium and grow in suspension at 27 °C.
3. After they have reached a concentration of $2-4 \times 10^6$ cells/ml (normally after 5–6 days) dilute with fresh SF-4 medium to 2×10^6 cells/ml and repeat this procedure. After two to three cycles the cells are adapted to serum-free growth.

b. Gradual medium replacement

1. Seed cells in a tissue culture flask or a Petri dish at a concentration of 5×10^5 cells/25 cm^2 surface in 3.6 ml serum containing medium. After 1 day add $^1/_{10}$ volume of SF-4 medium and grow the cells to 60 % confluence.
2. Subculture in 3 ml serum containing medium and SF-4 medium (9:1 v/v) and add after one day volume SF-4 medium.
3. Subculture in 3 ml serum containing and SF-4 medium (3:1 v/v) and add after one day. volume SF-4 medium.

2. Guidelines for specific growth conditions

a. Monolayer culture

Using a pipette aspirate medium and floating cells from a confluent monolayer, discard and again add about 4 ml of fresh complete medium to a 25 cm^2 flask. Resuspend cells by pipetting the medium across the monolayer. Observe cell monolayer to ensure complete cell detachment from the surface of the flask. Perform viable cell count on harvested cells (e.g. using trypan blue). Inoculate cells at around 1.2×10^6 cells/25 cm^2 flask. Return cultures to incubator (27 °C ± 0.5 °C). On day three post-planting aspirate the spent medium from one side of the monolayer and re-feed the culture with fresh medium gently added to the side of the flask.

b. Spinner

Recalibrate the spinner flasks using a graduated cylinder or volumetric flasks as a reference. Calibration is performed with the impeller apparatus removed from the vessel. Impeller mechanisms must rotate freely, do not allow contact with vessel walls or base. Avoid physical stress as most invertebrate cells are sensitive to physical shearing. Adjust the spinner mechanism so that paddles clear sides and bottom of the vessel (adjust prior to autoclaving). Four to six confluent 75 cm^2 monolayer flasks are needed to initiate a 100 ml culture (4–5 flasks for the spinner culture and one as a backup). Dislodge cells from the base of the flasks as described in a. (monolayer culture). Pool the cell suspension and perform a viable cell count. Dilute the cell suspension to approximately 3×10^5 viable cells/ml in complete medium. For culture volumes of 75–100 ml, use a 100 ml spinner vessel. For volumes of 150–200 ml, use a 250 ml vessel. Stock cultures should be maintained in a 150 ml culture in a 250 ml spinner vessel. The top of the paddles will be slightly above the medium, which provides additional aeration to the cultures. Atmospheric gas equilibration is accomplished by loosening the side arm caps on the vessels (about $^1/_4$ turn). Incubate spinner vessels at 27 °C ± 0.5 °C at a constant stirring rate of 75 rpm. Re-seed spinner cultures to approximately 3×10^5 cells per ml twice weekly in well-cleaned, sterile vessels. Once every two weeks spinner cultures may be gently centrifuged at 100 × g for 5 minutes and resuspended in fresh medium to reduce accumulation of cell debris and toxic metabolic by-products.

c. Shaker culture

The orbital shaker apparatus should have a capacity of up to 135 rpm. As standard flask use the 250 ml disposable sterile Erlenmeyer flask. The orbital shaker / flask assembly should be maintained in a 27 °C ± 0.5 °C non-humidified, non-gas regulated environment.

Aeration is accomplished by loosening the cap approximately $^1/_4$ turn (within the intermediate closure position). In this condition, there is no oxygen limitation to the cells and they therefore proliferate with maximal rates.

Inoculate a 250 ml Erlenmeyer flask with 100 ml of complete medium containing 3×10^5 viable cells per ml. Set the orbital shaker 125–135 rpm. Subculture to approximately 3×10^5 cells/ml twice weekly. Every three weeks, cultures may be gently centrifuged at 100 × g for 5 minutes and pellets resuspended in fresh medium to reduce accumulation of cell debris and toxic metabolic by-products. As cultures may be passage number dependent, fresh cultures should be established from frozen seed stocks every three months.

Guidelines for virus and recombinant protein production

Anchoraged cells

1. Seed the cells in a concentration of 2×10^6 cells / 25 cm^2 flask. Cell normally attach within 15 min but firmly after 1 h.
2. After 1 day remove the medium and incubate the cells for 1 h, with gentle rocking, with 0.5 ml of virus suspension containing $4-8 \times 10^7$ tissue culture infective dose 50 % (TCID50) units per cell, giving a multiplicity of infection of about 10–20 $TCID_{50}$ units per cell.
3. Wash the cells after removal of the medium and add 4 ml of fresh medium.
4. Isolate infectious virus, polyhedra or recombinant proteins from 48 h post infection onwards. After 72 h post infection, cell start to lyse.

Suspension cultures

1. Centrifuge cells of a suspension culture in logarithmic growth (500 g for 5 min).
2. Resuspend the cells to a density of 10^7 cells/ml in virus containing medium. This medium should contain $1-2 \times 10^8$ $TCID_{50}$ units of virus to give a final multiplicity of infection of 10–20 $TCID_{50}$ units per cell.
3. Isolate infectious virus, polyhedra or recombinant proteins from 48 h post infection onwards. After 72 h post infection, cell start to lyse.

Freezing

Prepare desired quantity of cells in either spinner or shaker culture, harvesting in mid log phase of growth at a viability of > 90 %. Determine the viable cell count and calculate the required volume of cryopreservation medium required to yield a final cell density of 0.5 to 1.0×10^7 cells/ml. Prepare the required volume of cyropreservation medium (7.5 % DMSO and 10 % BSA or FCS) in SF-4 Baculo Express medium. Hold

medium at +4 °C. Pellet cells from culture medium at 100 × g for 6 minutes. Re-suspend pellet in the determined volume of +4 °C cryopreservation medium. Incubate cell suspension at +4 °C for 30 minutes (until well chilled). Dispense aliquots of this suspension to cryovials. Frozen cells are stable indefinitely under liquid nitrogen.

Recovery

Recover cultures from frozen storage by rapid thawing a vial of cells in a 37 °C water bath. Transfer the entire contents of the vial into a 250 ml shaker flask containing 100 ml complete growth medium and incubate culture as described in shaker culture. Maintain culture between 3×10^5 and 1×10^6 cells/ml for the first two subcultures after recovery, and then returning to the normal maintenance schedule.

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Bluespotted stingray [*Neotrygon kuhlii*]
The Bluespotted stingray, is a species of stingray of the family Dasyatidae. The body is rhomboidal and colored green with blue spots. The Bluespotted stingray is commonly found in waters of depths above 90 m (295 ft), being commonly found in sand and mudflats, but is also encountered near rocky coral reefs and sea grass beds. The Great Barrier Reef is an area for high protection of Bluespotted stingray. A major threat to this stingray is the destruction of coral reefs.

Hard coral skeleton

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