Product: SF-4 Baculo Express ICM (Insect Culture Medium)
Cat. Nr.: 9-00F38
Ready-to-use, no supplementation required

Introduction - General information on insect cell culture

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 aminoacids, media for insect cell culture therefore contain high levels of aminoacids (Grace 1962, Weiss et al. 1981)

Organic acids:
Insect blood contains a unusually high level of free organic acids such as e.g. citrate, succinate, oxalate or malate and 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-4 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.

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 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).

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 frug*iperda (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 $2x10^5$ 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 $2\text{-}4x10^6$ 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 $5x10^5$ 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 ¼ volume SF-4 medium.

3. Subculture in 3 ml serum containing and SF-4 medium (3:1 v/v) and add after one day ¼ 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 x 10^6 cells/25cm^2 flask. Return cultures to incubator (27° ± 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 x 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 ± 0.5°C at a constant stirring rate of 75 rpm. Re-seed spinner cultures to approximately 3 X 10^5 cells per ml twice weekly in well-cleaned, sterile vessels. Once every two weeks spinner cultures may be gently centrifuged at 100 x 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 ± 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 x 10^5 viable cells per ml. Set the orbital shaker 125-135 rpm. Subculture to approximately 3 x 10^5 cells/ml twice weekly. Every three weeks, cultures may be gently centrifuged at 100 x 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 2x10^6 cells/25 cm2 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 x 10^7 tissue culture infective dose 50% ($TCID_{50}$) 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 (500g for 5 min).
2. Resuspend the cells to a density of 10^7 cells/ml in virus containing medium. This medium should contain 1-2x108 $TCID_{50}$ 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 x 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 x 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 x 10^5 and 1 x 10^6 cells/ml for the first two subcultures after recovery, and then returning to the normal maintenance schedule.

Literature:

Strauss A. et al (2003) Amino-acid-type selective isotope labeling of proteins expressed in Baculovirus-infected insect cells useful for NMR studies *Journal of Biomolecular NMR* **26** 367-372

Schlaeger E.J. (1996) Medium Design for insect cell culture;
Cytotechnology **20** 57-70.

Schlaeger E.J. (1996) The protein hydrolysate, Primatone RL, is a cost-effective multiple growth promotor of mammalian cell culture in serum-containing and serum-free media and displays anti-apoptosis properties; *Journal of Immunological Methods* **194** 191-199.

Schlaeger E.J., Foggetta, M., Vonach, J.M., Christensen, K. (1993) SF-1, a low cost culture medium for the production of recombinant proteins in baculovirus infected cells *Biotechnology Techniques* **7** (3) 183-188

Schlaeger, E.J., Loetscher, H., Gentz, R. (1992) Fermentation scale up: Production of soluble human TNF receptors . in Workshop on Baculovirus and recombinant protein production processes, Interlaken, Switzerland. Editors: J.M. Vlak, E.-J. Schlaeger, A.R. Bernard.

Ljunggren, J., Häggström, I. (1992) Glutamine limited fed-batch culture reduces the overflow metabolism of amino acids in myeloma cells. *Cytotechnology* **8** 45-56

Law, J.H., Wells, M.A. (1989) Insects as biochemical models. *J.Biol.Chem*. **264** 16335-16338

Grace, T.D.C. (1962) Establishment of four strains of cells from insect tissue grown in vitro. *Nature* **195** 788-789

Vaughn, J.L. (1968) A review of the use of insect tissue culture for the study of insect-associated viruses. *Curr.Top.Microbiol.Immunol*. **42** 108-128

Weiss, S.A., Smith, G.C., Kalter, S.S., Vaughn, J.L. (1981) Improved method for the production of insect cell cultures in large volume. *In Vitro* **17** 495-502