

Ready to use: Serum free culture in Insectomed SF express

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Serum free culture in Insectomed SF express 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* (e.g. D.Mel-2) cells.

No supplementation are required.

1 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.
- **Amino acids**
Insect blood contains a high level of aminoacids, media for insect cell culture therefore contain high levels of aminoacids.
- **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 mmol per insect.
- **pH, buffer and pH indicator**
Insect tissue fluids are more acidic and normally ranging from 6.2-6.9. 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. Insectomed SF 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 CO₂ is required to keep a constant range; no pH indicator is added. 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. Insect cell culture media therefore exhibit an osmolality of 340-390 mOsmol/kg compared to 290-330 for vertebrate cultures.
- **Glutamin, Glucose**
The excess metabolism of glutamine and glucose in mammalian cell culture results in an excess production of ammonium and lactate respectively, 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 Glutamin and Glucose can be used in insect cell culture media to support high cell densities growth.

2 Guidelines for adaptation to serum free Insectomed SF express

Insectomed SF express 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* (e.g. D.Mel-2) cells.

Special regimens are required to adapt insect cells from serum-containing to serum free Insectomed SF express: 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.

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 Insectomed SF express 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 medium to 2×10^6 cells/ml and repeat this procedure. After two to three cycles the cells are adapted to serum-free growth.

Gradual medium replacement:

1. Seed cells in a tissue culture flask or a Petri dish at a concentration of 5×10^5 cells/25 cm² surface in 3.6 ml serum containing medium. After 1 day add 1/10 volume of Insectomed SF express and grow the cells to 60 % confluence.
2. Subculture in 3 ml serum containing medium and Insectomed SF express (9:1 v/v) and add after one day 1/4 volume Insectomed SF express.
3. Subculture in 3 ml serum containing and Insectomed SF express (3:1 v/v) and add after one day 1/4 volume Insectomed SF express.

3 Guidelines for specific growth conditions

3.1 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 cm² 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 cm² flask. Return cultures to incubator (+27 ± 0.5 °C). On day three of 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.

3.2 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 cm² 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 3x10⁵ 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 3x10⁵ cells per ml twice weekly in well-cleaned, sterile vessels. Once every two weeks spinner cultures may be gently centrifuged at 100xg for 5 minutes and re-suspended in fresh medium to reduce accumulation of cell debris and toxic metabolic by-products.

3.3 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 3x10⁵ viable cells per ml. Set the orbital shaker 125-135 rpm. Subculture to approximately 3x10⁵ cells/ml twice weekly. Every three weeks, cultures may be gently centrifuged at 100xg 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.

4 Guidelines for virus and recombinant protein production

4.1 Anchored cells

Seed the cells in a concentration of 2×10^6 cells/25 cm² flask. Cells normally attach within 15 min but firmly after 1 h.

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 TCID₅₀ units per cell.

Wash the cells after removal of the medium and add 4 ml of fresh medium. Isolate infectious virus, polyhedra or recombinant proteins from 48 h post infection onwards. After 72 h post infection, cells start to lyse.

4.2 Suspension cultures

1. Centrifuge cells of a suspension culture in logarithmic growth (500xg for 5 min).
2. Resuspend the cells to a density of 10^7 cells/ml in virus containing medium. This medium should contain $1-2 \times 10^8$ TCID₅₀ units of virus to give a final multiplicity of infection of 10-20 TCID₅₀ units per cell.
3. Isolate infectious virus, polyhedra or recombinant proteins from 48 h post infection onwards. After 72 h post infection, cells start to lyse.

5 Freezing and recovery

5.1 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 $0.5-1.0 \times 10^7$ cells/ml. Prepare the required volume of cryopreservation medium (7.5 % dimethyl sulfoxide (DMSO) and 10 % bovine serum albumine (BSA)) in Insectomed SF express. Hold medium at +4 °C. Pellet cells from culture medium at 100xg 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.

5.2 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.

6 Details on Insectomed SF express

Parameters	Insectomed SF express
Cat. No.	F 8275
Unit	500 ml
Storage	+2 - +8 °C, keep dark, do not freeze
Raw material	serum-free, with L-glutamine
Use	Insect cells
Please note	for <i>in vitro</i> use

Insect cell media from Biochrom AG:

<http://www.biochrom.de/en/products/cell-culture-media/insect-cell-media/>