BI
Biological Industries

# TECHNICAL GUIDE

# BIOINSECT-1

## Serum-Free Medium for the Culture of Insect Cells

Cat. No.: 05-050-1
Store at: 4ºC

## Product Description

BIOINSECT-1 is a serum-free medium optimized for the culture of lepidopteran insect cells. The medium supports both suspension and stationary cultures of SF-9 cells derived from the pupal ovarian tissue of spodoptera frugiperda. SF-9 cells are suitable hosts for the replication of the baculovirus Autographa colifornica nuclear polyhedrosis virus. This virus, isolated from the Alfalfa looper, is used for the recombinant expression of heterologous proteins in the baculovirus expression vector system (BEVS). Insect cells, infected with this virus, display accumulations of the highly expressed protein polyhedrin, within the nuclea (polyhedra).

## Serum-Supplemented Cultures

Insect cells are incubated at 27-28ºC in an open environment. SF-9 cells are usually cultured in Grace's medium supplemented with TPB and yeastolate (TNM-FH medium) plus 10% heat-inactivated FCS. Cells may also be cultured in IPL-41 medium containing 10% heat-inactivated FCS. A stationary culture will reach confluency in 3-6 days. The cells are loosely attached to the substrate, and when the cells are subcultured, it is necessary to scrape the cells gently to remove them. To initiate a suspension culture, inoculate 3-5 x $10^5$ cells/ml from the stationary culture in a 250ml shaker flask, containing 100ml of complete medium. Subculture the cells to 3-5 x $10^5$ cells/ml twice weekly. The shaker should be maintained at 27-28ºC. The caps of the flasks should be loosened for aeration.

## Serum-Free Cultures

Either weaning of direct adaption may be used for transferring cells from serum-containing media to BIOINSECT-1 serum-free medium. We recommend using the weaning procedure for monolayer, as well as for suspension culture.

## Weaning Procedure

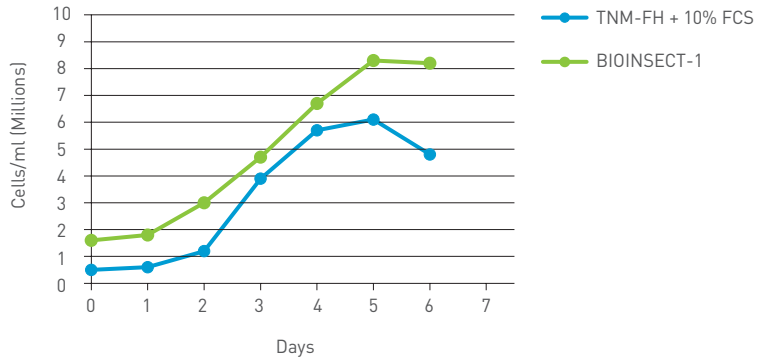
1. Transfer cells in the logarithmic phase from the serum-containing medium into 50% (v/v) mixture of serum-supplemented medium and BIOINSECT-1.
2. Subculture the cells after 3 days and reduce the percentage of the serum-supplemented medim to 40%.
3. Continue with the subculturing of the cells every 3 days and with each passage reduce the concentration of the serum-supplemented medium by a further 10%.
4. On the sixth passage, the cells will be fully adapted to BIOINSECT-1 serum-free medium.

## Maintenance of SF-9 cells in BIOINSECT-1 serum-free medium

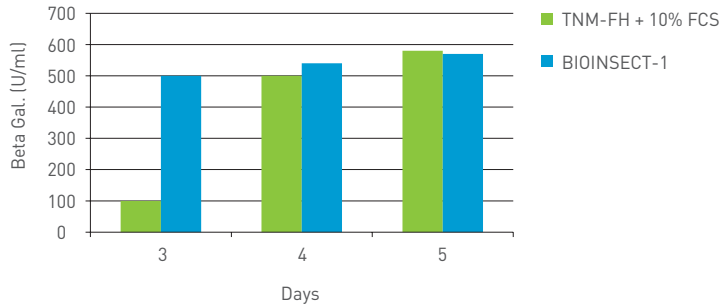
| | Stationary culture | Suspension culture |
|---|---|---|
| **Inoculation density** | 6-10 x $10^4$ cells/$cm^2$ | 1.5 x $10^6$ cells/ml |
| | 2-3 times/week | Every 3-4 days |
| **Subculture** | Subculture the cells when the viable cell count reaches 40-50 x $10^4$/$cm^2$, with greater than 90% viability | Subculture the cells when the viable cell count reaches 3-5 x $10^6$cells/ml, with greater than 95% viability. After 5 days in culture, the cell density reaches 6-8 x $10^6$ cells/ml. |

The culture may be gently centrifuged when subculturing, in order to remove the toxic by-products in the supernatant.

### SF-9 Cell Growth Curve




### Beta Galactosidase Expression: Suspension Culture




BI
Biological Industries
Culture of Excellence

Biological Industries Israel Beit Haemek Ltd.
Kibbutz Beit Haemek 2511500, Israel
T.+972.4.9960595 F.+972.4.9968896
Email: info@bioind.com
www.bioind.com

CE
CERTIFIED QMS ISO 9001 2008
CERTIFIED QMS ISO 13485 2003
cGMP Manufacturing Facility