

## **Sf21 cells in Insectomed SF express: growth pattern and infection with Baculovirus**

Biochrom AG Information

Biochrom AG's insect cell medium Insectomed SF express is suitable for the cultivation of *Spodoptera frugiperda* and *Drosophila melanogaster* cells. All cell types can be cultivated using Insectomed SF express. The complete medium contains all additives required for the cultivation of insect cells in ideal concentration: steroids, amino acids, organic acids, glutamine, and glucose. Insectomed SF express is suitable for the expression of recombinant proteins.

Growth rate and vitality of the cells in Insectomed SF express are comparable to other serum-free insect cell media. Several customers have already successfully tested Insectomed SF express for the cultivation of insect cells and do recommend the usage of the medium for infections with Baculovirus. During these tests, cells were successfully removed from the preculture into the new medium. The infection rate of Sf21 cells with the Baculovirus was nearly 100 per cent after three days. Please find below all the results in detail.

### **1 Insectomed SF express use**

Biochrom AG's new insect cell medium Insectomed SF express is ideally suited for the cultivation of *Spodoptera frugiperda* (Sf9, Sf21), BTI-TN-5B1-4 (High Five™) cells and *Drosophila melanogaster* (e.g. D.Mel-2) cells.

All cell types can be cultivated using Insectomed SF express: monolayer, spinner or shake cultures, adherent cells and suspension cells.

The complete medium is suitable for the expression of recombinant proteins.

### **2 Material and methods**

Material:

- Insectomed SF express (cat. no. F 8275, liquid medium, 500 ml bottles)
- incubator from New Brunswick Scientific company, model Innova44
- centrifuge from Eppendorf Deutschland company, no. 5810R
- safety cabinet class II from Heraeus company, model HS18, HeraSafe
- fluorescence microscope Eclipse TE2000-U from Nikon company
- Sf21 insect cells, DSMZ no. ACC119 (prior to the start of the experiment, the cells were cultivated in a siliconised shaking flask filled with Insectomed SF express for three weeks)
- virus: human-EPO-GFP (Baculogold from BD Biosciences)

**Methods:**

- culture volume: 10 and 30 ml
- culture period: 6 days
- virus stock with a TCID<sub>50</sub> (50% tissue culture infectious dose<sup>1</sup>) with a titer of  $2 \times 10^8$  viruses/ml
- growth rate identification and Epo-GFP (GFP: green fluorescent protein) expression

### 3 Experiment set-up

Three weeks prior to the start of the experiment, the insect cells were cultivated in Insectomed SF express in a serum-free manner. One day prior to the start of the experiment, the medium was completely changed; the cell count was determined and set appropriately to ensure that the cells are in the logarithmic growth phase at the moment of infection.

The cells were removed from the preculture before being centrifuged and resuspended in 30 ml and 10 ml medium respectively.

After 20 minutes, the particular virus suspension was added, with the cell count being determined and the respective MOI (multiplicity of infection<sup>2</sup>) being calculated (see table 1).

**table 1: initial conditions**

Insectomed SF express			
low cell count $7.1 \times 10^5$ / ml		high cell count $3.3 \times 10^6$ / ml	
control	MOI 4.2	control	MOI 4.0

The infected cultures were cultivated at 27° C for 6 days while being shaken. Samples were taken on a daily basis, and the cell counts were determined applying the trypan blue method by means of a Neubauer counting chamber. The infection rate was determined by enumerating the GFP-marked cells.

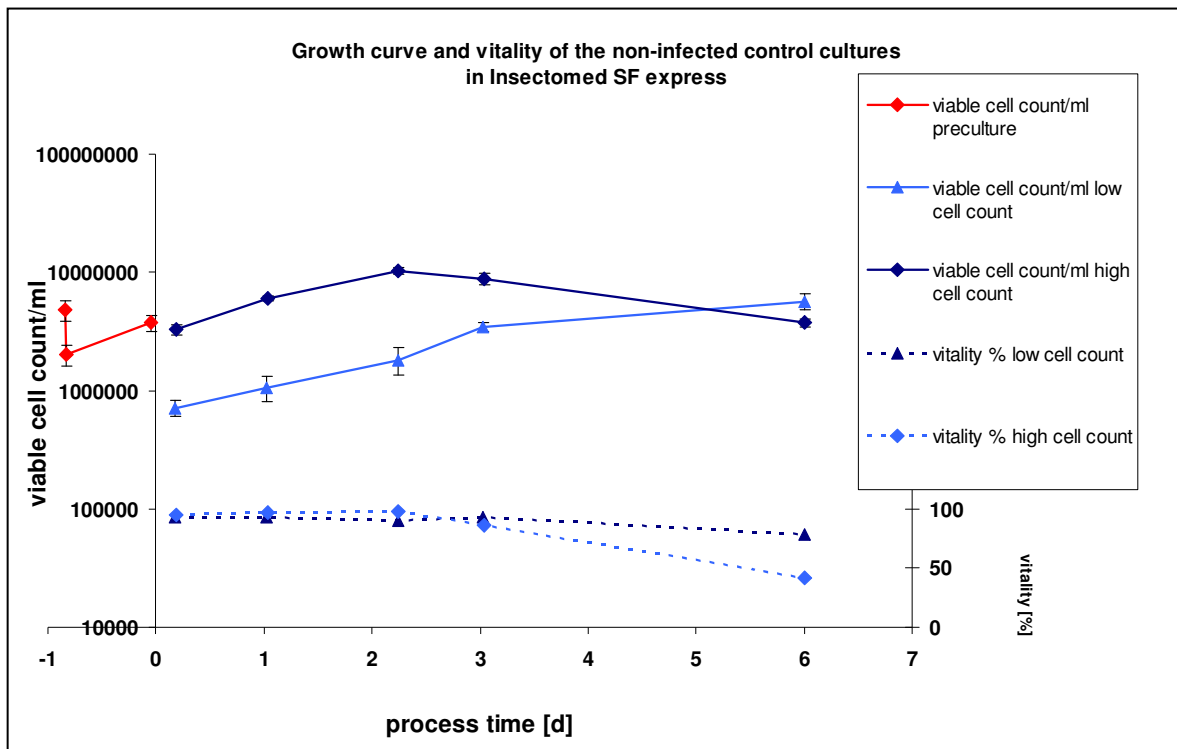
## 4 Results

### 4.1 Cell growth in Insectomed SF express

The cells could be removed from the preculture to Insectomed SF express without any difficulty, while they were continuously growing at the same division rate. The insect cells displayed an extreme adherent behaviour in Insectomed SF express.

<sup>1</sup> Virus titer application that leads to 50 % of the cells being infected.

<sup>2</sup> In the field of virology, MOI represents the numerical relation between virus and target cells.



**fig. 1: growth curve and vitality of the non-infected control cultures**

Initially, cell culture vitality is above 92 %. The rate declines to 80 % only after the maximum cell count in the system has been reached.

**table 2: growth rate**

parameter	Insectomed SF express
doubling time preculture (h)	21
doubling time culture (h)	30
viable cell count / ml	$1.0 \times 10^7$
vitality at the beginning (%)	92
vitality after 4 days (%)	80

For the cell line Sf-21, ACC 119, the DSMZ (*Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH*, the German Collection of Microorganisms and Cell Cultures) indicates a doubling time in serum-containing medium of approx. 3 days. In Insectomed SF express, doubling times of 21 h (without virus infection) and 30 h (after virus infection) are obtained.

## 4.2 Course of infection and infection rates

The growth curves of the infected cells proceeded as expected: cells infected with a MOI of 3 to 4 stagnate at their starting cell count level, with the culture entering the dying phase after two to three days.

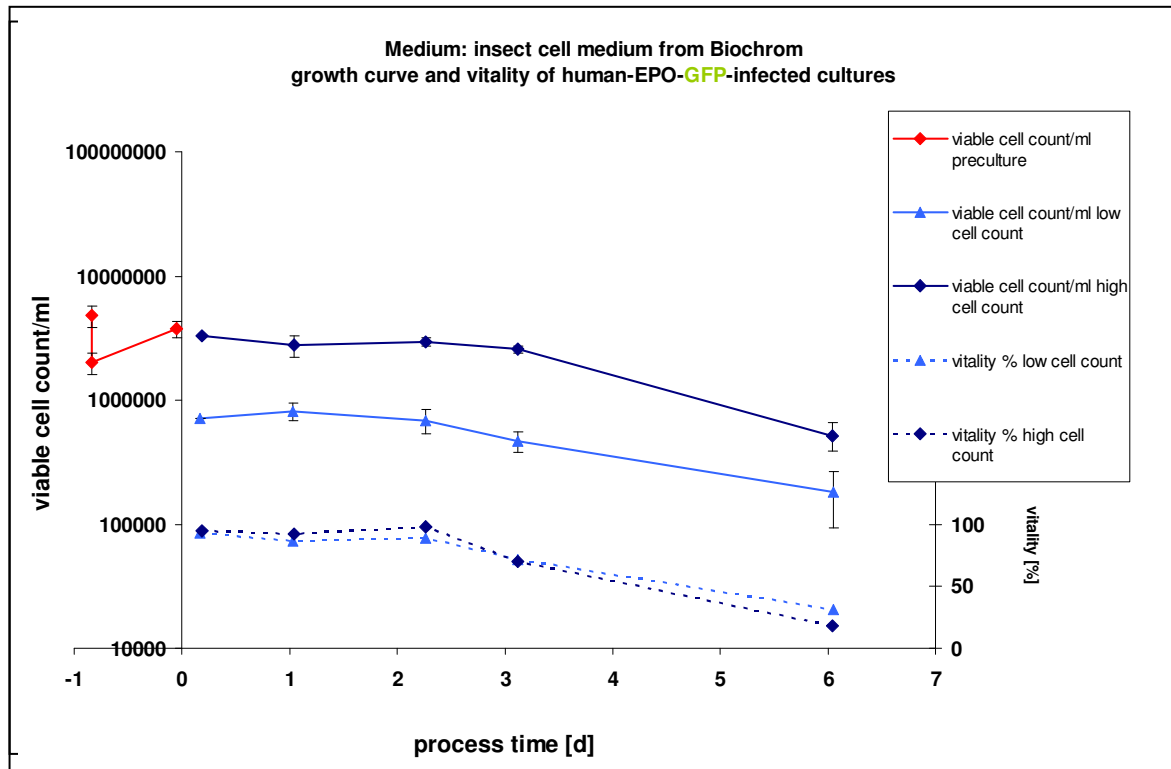


fig. 2: growth rate and vitality of human-EPO-GFP-infected cultures

A MOI of 3.5 results in an estimated primary infection of 97.0 %. After a 54-hour period, a value of 94 and 99 % was reached. Insectomed SF express produced high infection rates that were determined indirectly by means of EPO-GFP product formation.

table 3: infection rate at the moment of sampling

moment of sampling	infection rate in Insectomed SF express
24 h	61 and 65 %
54 h	94 and 99 %

### 4.3 Summary of the results

Growth pattern and infection follow the expected course. After 54 hours, a high percentage of cells were infected and produced EPO-GFP. The division rate amounting to 21 and 30 h respectively is significantly above the value indicated by DSMZ. This allows for a recommendation to use Insectomed for the cultivation and infection with Baculovirus.

## 5 Product details of Insectomed SF express

parameter	Insectomed SF express
cat. no.	F 8275
units	500 ml
storage	+2 - +8 °C
raw material	proprietary formulation; serum-free
use	medium for insect cells
note	<ul style="list-style-type: none"><li>➤ for <i>in vitro</i> use</li><li>➤ "ready to use"</li></ul>

- Notes for adapting the cells to Insectomed SF express:  
[http://www.biochrom.de/fileadmin/user\\_upload/service/Tipps\\_und\\_Hinweise/englisch/100521\\_hintergrundinformation\\_insektenzellmedium\\_en.pdf](http://www.biochrom.de/fileadmin/user_upload/service/Tipps_und_Hinweise/englisch/100521_hintergrundinformation_insektenzellmedium_en.pdf)
- **Our tip:** Use our new disposable counting chamber C-Chip to check the cell count in a fast and easy way. Please contact [info@biochrom.de](mailto:info@biochrom.de) to receive a free sample.

### Acknowledgement:

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