Cultivation of the insect cell line Sf9

Introduction
Insect cells are, in contrast to prokaryotic cells, able to produce similary eucaryotic proteins (i. e. post-translational modifications, protein folding etc.). The most commonly used cell line in biotechnology applications is type Sf9 (Spodoptera frugiperda ovary cells). These cells can be growth in suspension culture or adherent as monolayer. A variety of recombinant proteins can be obtained by utilizing the baculovirus expression system.

1. Equipment and Materials Used
- BIOSTAT® Aplus 2 L, CC-System
- Clean bench
- Microscope
- Counting chamber or cell counter
- Shaker (e. g. Certomat, Sartorius)
- 1 L inoculation flask
- 2 L Schott flask with stainless steel head plate and sterile filter
- STT-connectors
- Sterile filter 0.2 µm (e. g. Midisart® 2000)
- Sf9 cell line (ATCC No. CRL-1711)

2. Overview of setting-up procedures

a) Timetable
Day 1: Sterilization of equipment (inoculation flask, Schott flask etc.)
Day 1: Preparation & inoculation of preculture
Day 2: Cleaning, preparation & sterilisation of the culture vessel
Day 3: Calibration of DO probe and inoculation if cell density in preculture have reached the starting cell number

b) Bioreactor
- Cleaning of the culture vessel by incubation with 1% (w | v) NaOH
- Calibration and installation of the pH-electrode
- Installation of the pO_2 probe
- Washing with deionized water
- After this the vessel should be filled with deionized water to a level covering both probe membranes
- Connection of peripheral equipment (tubes and filter for air inlet & exhaust, sampling device, STT connector for medium transfer etc.)
- Sterilization of the culture vessel by autoclaving according to the manual
- Transfer the medium from a sterile 2 L Schott flask with head plate and STT connection
- Calibration of the DO probe at cultivation temperature and cultivation mixing speed.

c) Medium
For cultivation of Sf9 cells Grace medium is recommended. TNM-FH medium is fully supplemented Grace's medium including trace metals, lactalbumin hydrolysate, yeastolate, and 10% heat inactivated fetal bovine serum. Transfer 2 L sterile 1× TNM-FH medium under sterile conditions into the culture vessel by hydrostatic pressure using the 2 L Schott flask and the STT connection.

Note
After sterilization all manipulation on the culture vessel should be carried out under "clean bench" conditions.

d) Inoculum
Inoculum is prepared in a sterile 1000 mL inoculation flasks containing 200 mL sterile 1× TNM-FH. Transfer the Sf9 cells in the flask and incubate the culture at 28°C and 90 rpm in a thermo shaker. For inoculation the culture vessel an initial cell density of 2×10^5 cells should be provided.

e) Recommended culture conditions

Culture volume:	2 L
Aeration:	0.05–0.1 vvm
Stirrer:	50–200 rpm
Temperature:	28°C
DO:	60%, controlled
pH value:	6.3

3. Analytical Procedures
Cell counting with the trypan blue method.

The trypan blue method based on the characteristic of living cells to reject the blue dye. In contrast dead cells assimilate the dye and can be directly observed under the microscope.

Cell counting is carried out as follows:
Mix 10 µl of Trypan blue with 10 µl cell suspension. Use 10 µl of this mixture for microscopic counting in a counting chamber (e. g. Neubauer, Thoma) with minimum of 100 × magnification.

Calculate cell viability as follows:

Viable count = unstained cells × CSF X DF

With: CSF = Chamber specific factor
DF = Dilution factor

Determination of viable cells:

$$\text{Viability} = \frac{\text{unstained cells}}{\text{unstained cells + stained cells}} \times 100\%$$

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