

Continuous Recombinant Protein Production in Baculovirus Infected SF9 Cells using CELLine *classic* 1000 Two-Compartment Bioreactors

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Introduction

In recent years, baculovirus expression vectors (BEV) systems have become an efficient and popular method for the production of recombinant proteins in insect cell lines. The system allows to achieve relatively high levels of heterologous gene expression and the posttranslational modifications of the gene products are closely parallel to the modifications within mammalian cells. Recombinant proteins expressed in the cytoplasm of an insect host cell often reach concentrations of up to 1 mg/ml. In contrast, secreted proteins are usually only found to be present at concentrations below 0.01 mg/ml. Therefore milligram-amounts of a secreted protein can only be obtained by handling several litres of cell culture volume using either a small scale bioreactor or handling large numbers of standard cell culture disposables. As a consequence such experiments can become time consuming and cost intensive.

The CELLine Two-Compartment Bioreactors are designed to ensure an optimal nutrient and oxygen supply of the cells and thereby allow the cultivation of eukaryotic cells to densities which are 50 times higher than compared to standard homogenous cell culture vessels. This protocol describes the cultivation of SF9 cells in a CELLine *classic* bioreactor to a density of 5 – 10 x 10⁷ cells/ml. The culture can be continuously maintained over several weeks and cells can be harvested every sixth day. Upon transfection of the SF9 cells with a BEV, this high cell densities drive the accumulation of recombinant protein titers which are between 1 to 2 magnitudes higher than in a standard homogenous cell culture vessel. The method was designed to minimize the necessary handling time and to allow significant cost savings, mainly resulting from a 95% reduction of the required serum supplementation. In addition, the condensed harvesting volumes prevent a laborious concentration of culture supernatants and the high specific product concentrations facilitate the protein purification from the contaminating serum background.

Operating Procedures

A. Preculture

Cultivate SF9 cells in 225 cm² Tissue Culture Flask to a density of 1x10⁶ cells/ml in 50ml SF900 II¹ supplemented with 10% FCS.

B. Continuous Cultivation of SF9 cells

Resuspend the 5x10⁷ cells obtained from the preculture in 15 ml of fresh medium supplemented with 10% FCS and inoculate the cell compartment of a CELLine *classic* 1000 bioreactor² (cultivation reactor) with the suspension. Add 1000ml of fresh basal medium (not supplemented with FCS) to the medium compartment.

Incubate the CELLine Reactor for 6 to 7 days at 28°C.

Harvest the cells from the cell compartment at a density of around 5 x 10⁷ cells per ml and remove

90% (about 17 to 18 ml) for baculovirus transfection (see paragraph C).

Spin down the remaining 10% (2 to 3ml) of the cells, resuspend them in 15 ml fresh medium with 10% FCS and continuously cultivate the cells as described above.

Perform subsequent harvests every 4 to 6 days. SF9 cultures can be continuously maintained over 2 to 3 months.

C. Baculovirus Transfection and Recombinant Protein Production

Spin down the cells harvested from the cultivation reactor (see paragraph B) and resuspend them in 15 ml of fresh medium containing 10% FCS

Add Baculovirus with an MOI 2 (around 15 x 10⁸ virus particles) into the cell compartment of a second CELLine *classic* 1000 bioreactor (production reactor) and subsequently add the prepared cell suspension.

Add 1000ml of fresh medium without FCS to medium compartment of the production reactor.

Incubate the infected cells for 4 to 5 days at 28°C.

Harvest the culture supernatant and/or cells from cell compartment containing your protein of interest.

The production reactor can be used continuously for several transfections of SF9 cells with the same BEV³.

Notes

¹ Best Results were obtained with SF900 II medium (Invitrogen), as alternative standard Grace's Insect Medium can be used.

² In order to obtain optimal performance, the semi-permeable membrane of the CELLline bio reactor needs to be equilibrated for 5 minutes prior to inoculation by the addition of 25 ml of basal medium to medium compartment.

³ In order to prevent protein precipitations to damage the semi-permeable membrane between the cell and the medium compartment, we recommend to store the CELLline bioreactor in 1x PBS in between two BEV infection cycles.