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eSf9™ Medium

Instructions for Use

Introduction

eSf9™ Medium is a chemically-defined, non-animal origin formulation developed to maximize the production of baculovirus from Sf9 cells and to provide robust performance in bioreactor production systems. eSf9™ Medium contains L-glutamine and does not require serum supplementation.

I. Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. Upon arrival, store the bottle of medium at 4°C to 8°C protected from light.
3. When not in use, store eSf9™ Medium at 4°C to 8°C protected from light.
4. When in use, protect eSf9™ Medium from light.

Suggested Materials

- Sf9 cells (minimum of 20 million viable cells)
- eSf9™ Medium (Lonza P/N: BP12-931Q)
- 125 mL Erlenmeyer flasks
- 70% v/v ethanol or isopropanol
- 100 mL conical tubes
- 50 ml centrifuge tubes
- 100-400 g/L sterile filtered glucose (optional)
- 200mM L-glutamine (Lonza P/N: 17-605E)

II. Instructions for Use

Initiation of Culture Process

1. Aliquot 80 ml of eSf9™ Medium into a 100 ml conical tube wrapped in foil to protect the medium from light and warm to room temperature (RT, 20°C to 25°C) before use.
2. Wipe the cryovial containing the eSf9 cells with 70% v/v ethanol or isopropanol before opening. In a Biological Safety Cabinet (BSC), briefly twist the cap a quarter turn to relieve pressure, and then re-tighten. Quickly thaw the cryovial in a 37°C water bath.
3. Remove the cryovial promptly before the last crystal of ice melts. Do not submerge the cryovial completely. Thawing the cells for longer than 1½ minutes may result in less than optimal cell viability.
4. Dry the cryovial with a lint-free wipe, spray with 70% v/v ethanol or isopropanol, and then wipe to remove excess.
5. Transfer the cryovial to a Biological Safety Cabinet. Using a micropipette, gently add the thawed cell suspension to a 50 ml centrifuge tube containing 30 mL of RT of eSf9™ Medium.
6. Gently mix the cell suspension by pipetting up and down 3 to 5 times.
7. Centrifuge the cell suspension at 300 x g for 5 minutes at RT. Carefully aspirate the supernatant and resuspend the cell pellet with 1mL of RT eSf9™ Medium.
8. Gently mix the cells by pipetting up and down.
9. Add 20 mL of RT eSf9™ Medium to the conical tube. Count the cells with a hemacytometer or cell counter and calculate the total number of viable cells.
10. Transfer 20 million viable cells to a 125 mL Erlenmeyer flask, and bring the total volume to 25 mL with RT eSf9™ to achieve a target density of 0.8 million cells per mL.
11. Incubate the cell stock at 28°C ± 0.5°C, for 72 to 96 hours at 105 to 120 rpm agitation on a 10 mm orbital shaker.

III. Subculturing Sf9 Cells

1. Aliquot 25 mL of eSf9™ Medium into an empty Erlenmeyer flask wrapped in foil and warm to RT.
2. Label the flask with the Sf9 cell information, date, and passage number. Remove the cell stock Erlenmeyer flask from the incubator and homogenize the contents of the flask by gently rocking forward and backward.
3. Count the cells with a hemocytometer or cell counter and calculate the total number of viable cells.
4. Calculate the cell suspension volume needed to inoculate the new Erlenmeyer with 500,000 cells/ml (12,500,000 total cells).

$$\text{Volume of cells} = \frac{\text{Final volume (mL) x Target Density (cells/mL)}}{\text{Cell Density (Viable cells/mL)}}$$

5. Remove the same volume of medium from the new Erlenmeyer flask as the volume of cell suspension calculated in step 4.
6. Add the calculated cell suspension from the cell stock Erlenmeyer flask to the new Erlenmeyer flask.
7. Return the new Erlenmeyer flask to the 28°C ± 0.5°C, incubator for 72 hours (recommended infection time) to 168 hours (for peak cell density) at 105 to 120 rpm agitation.
8. After 96 hours of culture, sample each culture for glucose, L-glutamine, and any other desired metabolites.

   Supplement cultures with additional glucose to prevent glucose depletion. For example, if cultures are sampled every other day, when glucose concentrations are below 4 g/L, supplement up to 7 g/L.

   Supplement cultures with additional L-glutamine to prevent L-glutamine depletion. For example, if cultures are sampled every other day, when L-glutamine concentrations are below 4 mM, supplement up to 8 mM.

IV. Viral Infection of Sf9 cells by Baculovirus

Follow section III. Subculturing Sf9 cells to prepare the cells for infection.

Viral infections should be carried out at a cell density between 3-4 million using a Biological Safety Cabinet and following all necessary safety precautions. Infections are not typically carried out until the cells are fully adapted to the medium (**at least 10 population doublings from thaw**).

The recommended multiplicity of infection (MOI) for Baculovirus production is 0.1 MOI.

1. Remove the Erlenmeyer flask containing the cells from the incubator.
2. Homogenize the contents of the flask by gently rocking forward and backward.
3. Count the cells with a hemacytometer or cell counter and calculate the total number of viable cells.

   Determine the amount of virus required for the infection using the following formula. The optimum multiplicity of infection (MOI) for Baculovirus production is 0.1 MOI. Total Cell # = cell count x 25ml

$$\textit{Virus Stock Required (mL)} = \frac{\textit{Total Cell \# x MOI}}{\textit{Virus Titer}}$$

   If the volume of virus stock is less than 1 mL then dilute with eSf9™ Medium to 1 mL final volume.
4. Infect the cells by adding the infection mix to the Erlenmeyer flask containing the cells.
5. Incubate the infected cells in the Erlenmeyer flask at 28°C ± 0.5°C, for 72 hours on an orbital shaker at 105 to 120 rpm agitation.
6. Harvest the cells at the end of the 72-hour incubation by transferring the culture into a 50 mL conical tube.
7. Centrifuge the cell suspension at 300 x g for 10 minutes at RT.
8. Collect and filter the virus-containing supernatant using a 0.2 μm PES filter unit.
9. The filtered supernatant can be stored at 4°C for a week or at -80°C in a 20% sucrose solution for longer periods of time (up to 10 years).

Product Use Statement

These products are to be used for further manufacturing or laboratory purposes only. Performance characteristics have not been established.