Protein Science - PEL-00006 Baculovirus Protein Expression

Purpose

To describe the procedure for baculovirus protein expression.

Materials and Equipment

- Cedex HiRes automated cell counter
- Innova 44 incubator shaker
- Beckman J6-MI centrifuge
- JS-4.2 rotor
- Biological safety cabinet
- Serological pipette
- P1000 pipette
- P1000 pipette tip
- Sf-900 III Serum-Free Media (Thermo Fisher, cat. #12658001)
- 5 mL sterile serological pipette
- 10 mL sterile serological pipette
- 25 mL sterile serological pipette
- 50 mL sterile serological pipette
- Cedex HiRes Sample Cups
- Titered baculovirus stock
- Thomson 1.6 L Optimum Growth Flask (Thomson, cat. #931113)
- Dry ice

Procedures

A. Preparation

1. Warm Sf-900 III Serum-Free Media to room temperature.
2. Remove the seed culture from 27°C and place it into the biological safety cabinet.
3. Aseptically remove 1 mL of culture for cell concentration determination.
4. Using the procedure in PEL-00005, determine cell count and viability using the Cedex HiRes automated cell counter and record the values below.

Cell Count:________ Viability:________ Cell Size:________

5. Calculate the amount of culture needed to set cells at 6.5×10^5 cells/mL (24 hours prior to infection).

(6.5×10^5)(Desired Volume) / (Cell Count from A.4) = ________ mL of culture

6. Calculate the required amount of fresh Sf-900 III media.

Desired Volume – Volume Calculated in A.5 = ________ mL of Sf-900 III

7. Aseptically transfer the calculated amount of Sf-900 III to the appropriate vessel based on the total volume. (See the table below for vessel selection.)
8. Swirl the seed culture to ensure cell culture density is homogenous, then aseptically transfer the calculated amount of cell suspension to the flask from A7.
9. Place the seeded cell culture into the Innova 44 incubator shaker and set it to shake at 27°C at 125 rpm for at least 24 hours.

Volume Range	Growth Flask	1” Shake (rpm)	2” Shake (rpm)
20–25 mL	125 mL non-baffled (Corning)	140	N/A
30–50 mL	250 mL non-baffled (Corning)	140	N/A
80–100 mL	250 mL Optimum Growth Flask	185	125
150–200 mL	500 mL Optimum Growth Flask	185	125
700–900 mL	1.6 L Optimum Growth Flask	185	125
1–1.2 L	2.8 L Optimum Growth Flask	N/A	125
2 L	5 L Optimum Growth Flask	N/A	125

B. Infection

1. Remove seeded flasks from the 27°C incubator shaker and place them in the biological safety cabinet.
2. Aseptically remove 1 mL of culture from each flask for cell concentration determination.
3. Using the procedure in PEL-00005, determine the cell count and viability using the Cedex HiRes automated cell counter and record the values below.

Cell Count:________ Viability:________ Cell Size:________

4. Using the calculation below, determine the amount of titered baculovirus to add to each infection.

((Cell Count from B.3) × (Volume of Culture) × (Desired MOI)) / (Viral Titer) = mL of Virus Needed

5. Aseptically add the amount calculated in B.4 to each flask (and record the amount and the lot number of the virus stock).
6. Transfer the cultures to a 21°C incubator shaker and set them to shake at the appropriate speed for the culture vessel and shaker platform.

C. Harvest

1. After 72 hours of incubation, remove cultures from the 21°C incubator shaker and place them in the biological safety cabinet.
2. Aseptically remove 1 mL of culture from each flask for cell concentration determination.
3. Using the procedure in PEL-00005, determine the cell count and viability using the Cedex HiRes automated cell counter and record the cell counts, cell viability, cell size, cell diameter, and percentage of cell size shift.
4. In the biological safety cabinet, add culture to the appropriate centrifuge vessel (a 50 mL conical tube or a 250 mL or 500 mL conical centrifuge bottle) for the size of the culture.
5. Centrifuge the culture at 2,500 rpm for 15 minutes at 4°C.
6. Transfer the centrifuge container to the biological safety cabinet.
7. If the protein being expressed is secreted, decant the supernatant into an appropriately sized sterile vessel with a sealing cap and repeat steps C.4–C.7 until all of the culture has been centrifuged. Dispose of the centrifuge bottles in a biohazard waste bin.
8. If the protein being expressed is intracellular, decant the supernatant into the Wescodyne waste container in the biological safety cabinet and repeat steps C.4–C.8 until all the culture has been centrifuged.
9. Once the culture has been completely centrifuged, seal the centrifuge containers, remove them from the biological safety cabinet, and freeze them on dry ice for a minimum of 30 minutes.
10. Once the pellet has been fully frozen, transfer it to –80°C storage.