Technical Note:

Preparing a Suspension Culture of Sf9 and Sf21 Insect Cells in a Spinner Flask

Introduction

A basic protocol is provided below to enable the transfer of adherent Sf9 and/or Sf21 cells from tissue culture flasks into a suspension culture in Bellco spinner flasks. This procedure allows for the initiation and maintenance of suspension cultures of Sf9 and Sf21 cells.

Materials required

- Adherent Sf9 or Sf21 insect cells
- Culture medium, with serum or serum free.
- Spinner flasks Bellco #1965 Series
- Magnetic stir plate
- 26–28℃ incubator, CO2 not required

Procedure

When initiating suspension cultures of Sf9 or Sf21 cells, log phase cells of 95% viability or greater should be used to ensure a successful initiation process.

<u>To rapidly initiate a suspension culture of Sf9 or Sf21cells:</u>

- Grow up enough log phase adherent cells to start a spinner flask of the desired size with 0.5×10^6 viable cells/mL.
  - **Note**: Seeding at lower cell densities can cause increased doubling times during the initial stages of culture.
  - **Note**: Bellco recommends starting with a 100-mL or 250-mL spinner flask as these require fewer total cells than larger spinner flasks to initiate suspension cultures.

- Prepare clean, sterile spinner flask(s) with one of the following media types:
  - Grace's Insect Medium, Unsupplemented, plus 10% FBS.
  - Grace's Insect Medium, Supplemented, plus 10% FBS.
  - Serum Free Media
    - Gibco Sf-900 II SFM
    - Gibco Sf-900 III SFM
  - The recommended working volume of media is dependent on the size of the spinner flask, see below for recommended volumes based on maximizing aeration/oxygenation of the culture.
    - 100mL flask. Minimum working volume 30mL. Maximum working volume 50mL.
    - 250mL flask. Minimum working volume 80mL. Maximum working volume125mL
    - 500mL flask. Minimum working volume 200mL. Maximum working volume 250mL

- Detach cells from the tissue culture flask(s) and count cells to determine cell density and viability. Cell viability needs to be >95% to ensure successful adaptation.

- **Note:** Cells can typically be easily detached using the sloughing method which dislodges the cell monolater in the T-flask with the least manipulation and mechanical force which results in higher cell viabilities than other methods.
- **Basic sloughing method.**
    - Pour off to waste all but 5mL of media from the flask, irrespective of flask size.
    - Tilt the flask on its end so that all remaining media flows to one corner of the flask and away from the cells.
    - Draw some of the remaining media into a sterile pipette and stream the media across the cells starting at the bottom corner of the flask
    - It is recommended to use a side-to-side streaming motion and to move from the bottom corner up to the top opposite corner of the T-flask.
    - Always use a gentle media stream to dislodge the cells.

- Seed a clean, sterile spinner flask of the desired size with enough cells to reach a density of 1×10^6 cells/mL.
    - **Example**: A 100-mL spinner with 50 mL of culture at 0.5×10^6 viable cells/mL would require a total of $(0.5 \times 10^6$ cells/mL) × (50 mL) = 2.5×10^7 viable cells.

- Incubate the spinner flask(s) at 26–28℃ with constant stirring at 80–90 rpm.

- When the cells reach a density of about 2×10^6 to 4×10^6 cells/mL, passage them at a seeding density of between 3×10^5 to 5×10^5 viable cells/mL into appropriately sized flask(s).

References

1. Bellco Technical Report TN-2025-001. General Recommendations for Using Bellco Spinner Flasks for Insect Cell Suspension Cultures.
2. Bellco Technical Report TN-2025-002. Key Points to Consider in Spinner Flask Cell Culture.
3. Gibco User guide MAN0000030. Growth and Maintenance of Insect Cell Lines. Rev C.0

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