Technical Note:

General Recommendations for Using Bellco Spinner Flasks for Insect Cell Suspension Cultures

Introduction

Development and maintenance of a robust adherent cell line prior to establishing spinner flask cultures is essential. Sf9 and Sf21 cells can generally be thawed directly into adherent or suspension cultures due to high cell densities provided.

- **Note**: Some insect cell lines must be initiated into culture as adherent cells, expanded, and then adapted to suspension culture.

General Recommendations

- **Monitor and record cell growth.** Cells should be monitored daily through cell counts and viability. Bellco recommends that this data is maintained in a log or chart to troubleshoot potential issues that may arise.
- **Renew cultures periodically.** As a general rule, a new culture should be initiated from frozen stocks at least once every 3 months as cells begin to lose their infectivity and are no longer optimal for use in protocols. Depending on the culture conditions and the number of passages per week it may be required to start a new culture from frozen stock more frequently.
- **Maintain mid-log phase cell density.** When Sf9 and Sf21 cells reach a density of 2×10^6 to 4×10^6 cells/mL, they should be passaged at 3×10^5 to 5×10^5 cells/mL.
- **Use a surfactant to decrease cell shearing.** A 0.1% final solution of Pluronic™ F-68 is recommended for spinner flask cultures. Pluronic™ F-68 is a surfactant that helps reduce cell membrane shearing due to impeller forces.
    - **Note:** Some serum-free media may already contain Pluronic™ F-68 or other suitable surfactants in which case additional surfactants are not required.
- **Media Change.** It is not necessary to change the culture medium when culturing insect cells in suspension. Regular subculturing requires the removal of cell suspension and the addition of fresh medium based on the recommended seeding density of the cells. This addition of medium is sufficient to replenish cell nutrients.

Spinner Flask Recommendations

- **Spinner flask selection.** Bellco recommends using a spinner flask with a vertical impeller rather than one with a hanging stir-bar assembly for insect cells. A vertical impeller design provides better aeration at slower speeds which is optimal for reducing cell aggregation, maximizing aeration, minimizing shear related stress and optimizing cell viability.
- **Optimizing impeller speed.** Impeller speed needs to be optimized based on the design of the impeller blade used. Start at the lowest speed and incrementally increase until the speed is optimized to achieve the correct doubling time (approx. 24 hours for Sf21 cells and 48-72 hours for Sf9 cells), good cell viability, and low cell aggregation.
- **Impeller rotation.** The impeller should be rotating smoothly without any jerkiness or jumping motion. Smooth impeller motion is essential for reducing shear stresses, optimal aeration and high cell viabilities.

- **Impeller position.** The impeller must be submerged 1.0 cm or more into the media to ensure adequate aeration of the culture.
- **Recommended working volume**. The total culture volume in a spinner flask should not exceed one half of the indicated volume of the flask for proper aeration.
    - 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
    - 1000mL flask. Minimum working volume 300mL. Maximum working volume 500mL
    - 3000mL flask. Minimum working volume 900mL Maximum working volume 1500mL
- **Culture initiation**. Initiating a culture in spinner flasks larger than 500 mL is not recommended. Bellco recommends starting from smaller spinner flasks and gradually adapting the culture to larger size flasks.
    - **Note:** The impeller speed may need to be optimized when moving to different size flask(s).

Operational Recommendations

During operation

1. All caps on a spinner flask should be finger-tightened when incubating cell cultures. Loose caps are not required for aeration and can be a potential source of contamination.
2. Always open spinner flasks under a laminar flow hood when opening spinner flasks to passage cells, remove or add media. Always practice good sterile techniques with all manipulations.
3. Maintain sterility and avoid contamination by not dripping media or touching any instruments, for example. pipettes into or around the arm of the spinner flask without touching the sides when removing or adding media, taking samples etc.

Autoclaving

1. Ensure all rings are tightened around the spinner bar and the top of the flask prior to autoclaving. Moisture that can get into these spaces can be a cause of contamination.
2. The caps on the side arms of the spinner flask should be loose during the autoclaving cycle to ensure steam access to the flask and prevent pressure or vacuum build up in the flask during the autoclave cycle.
3. Autoclave the spinner flask vertically, DO NOT lay the flask on its side.
4. Autoclave at least twice when using the spinner flask for infections with baculovirus constructs (e.g., high-titer stocks, protein expression). Minimum cycle is at 121°C for 30 minutes. One wet cycle and one dry cycle is the minimum required to ensure proper sterility. Baculovirus particles can survive one autoclave cycle and cause contamination of subsequent cultures.

Cleaning

1. Do not use soap or detergents to clean the spinner flask, as residue will always remain on the surface and will cause cell mortality**.**
2. Wash with a 1% solution of 7X glass cleaner (Bellco part number 4782-10001) or with 10% acetic acid for at least 2 hours while spinning.
3. 7X glass cleaner is preferred over acetic acid as any trace elements of 7X are not detrimental to cell growth.
4. Acetic acid can inhibit cell growth if not completely rinsed after cleaning.

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