IS BAC™

Catalog No 9194 **1L**
Serum-free Medium for Insect Cell Culture

INTENDED USE

IS BAC™ is intended for further manufacturing use.

PRODUCT DESCRIPTION

IS BAC is a serum-free medium that supports the high density, suspension growth of *Spodoptera frugiperda (Sf9, Sf21)* and *Trichoplusia ni* (BTI-TN-5B1-4 or High-Five™) cells. This medium supports long term and high density suspension cell growth as well as high levels of baculovirus production and recombinant protein expression. Cells grown in other serum-free media can be subcultured directly into IS BAC with minimal adaptation.

FORMULA

IS BAC Medium is a complete, ready-to-use medium formulated with 10 mM L-glutamine. No additonal supplements (i.e. Pluronic F-68) are required. This medium does not contain antibiotics or antimycotics.

STORAGE AND STABILITY

IS BAC should be stored at 2°C to 8°C and protected from fluorescent light. Do not use after the expiration date printed on the label.

Indication of Deterioration:
Do not use if solution is cloudy or contains precipitates.

PRECAUTIONS AND WARNINGS

Do not use any bottle of liquid medium which shows evidence of particulate matter or cloudiness. Minimize exposure to light for all liquid components.

DIRECTIONS FOR USE

Insect cells currently adapted to serum-free media can be subcultured directly into IS BAC with essentially no adaptation (direct adaptation). Cells should be cultured for a minimum of three (3) passages in IS BAC to verify adaptation. When completely adapted to serum-free conditions, the cells will reach densities in excess of 6 X 10^6 cells/mL. Cells should be subcultured two times per week when the density reaches 2-4 X 10^6 cells/mL with at least 90% viability, to a subculture density of 3 X 10^5 cells/mL. If the direct adaptation method gives suboptimal performance, sequential adaption may be required. Additionally, frozen stock cultures of cells adapted to serum-free conditions can be directly recovered in IS BAC and should be grown for a minimum of three (3) passages in IS BAC. If cells are currently maintained in medium containing serum, sequential adaptation to serum-free medium is required. Use only plastic disposable Erlenmeyer flasks for shake cultures. Use of glass shaker flasks may result in cell death.

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A. Direct Adaptation

1. Suspension cell cultures adapted to growth in a serum-free medium can be directly subcultured into IS BAC, at a starting density of 3-5 X 10^5 cells/mL. It is important that cells be in the logarithmic phase of growth with at least 85% viability.

2. If the direct adaptation method gives suboptimal performance, use the sequential adaptation method.

B. Sequential Adaptation

1. Insect cell cultures grown in 5%-10% FBS supplemented medium or serum-free medium to a density of 1-3 X 10^6 cells/mL should be subcultured to a density of 3 X 10^5 cells/mL into a 50:50 ratio of IS BAC serum-free medium and the original growth medium. Also maintain an additional flask with the initial (first stage) culture conditions in the event that the cells do not adapt to the next stage in stepwise reduction of serum.

2. Incubate accordingly at 28°C until the viable cell density exceeds 2 X 10^6 cells/mL and viability is greater than 85%.

3. Next subculture to a density of 3 X 10^5 cells/mL into a 75:25 ratio of serum-free IS BAC medium and the original growth medium.

4. When the viable cell density reaches 2-3 X 10^6 cells/mL, with greater than 85% viability, subculture to 3 X 10^5 cells/mL into 100% IS BAC serum-free medium. Higher seeding densities (5 X 10^5 cells/mL) may be required for the first few passages in IS BAC alone.

5. Maintain cells in IS BAC for several passages, subculturing twice weekly to allow for complete adaptation.

6. Continue to subculture twice weekly when the density is between 1-3 X 10^6 cell/mL and viability is at least 90%.

7. If the cells do not survive at any one particular stage of adaptation, maintain the cells for an additional passage in the previous stage medium ratio before subculturing into the next stage.

CRYOPRESERVATION

Viable cell banks may be prepared by freezing cells in 93% cold IS BAC Medium + 7% DMSO. No proteins or other additions are required.

Freezing

1. Use cultures that are in logarithmic growth (2-3 X 10^6 cells/mL) with high viability (>85%).

2. Determine the viable cell density and prepare the required amount cryopreservation medium to freeze cells at final density of 1-2 X 10^7 cells/mL of cryopreservation medium.

3. Prepare sterile freeze medium by combining 93% cold IS BAC and 7% DMSO.

4. Harvest and centrifuge the cells at 200 X g for 5 minutes. Remove the supernatant from the tube without disturbing the cell pellet.

5. Gently resuspend the cells in cold cryopreservation medium to a denisty of 1-2 X 10^7 cells/mL.

6. Dispense 1-2 mL of this cell suspension into sterile cryovials.

7. Freeze the vials at a rate of 1°C/min. using a programmable cooler (or other standard method).

8. For long-term storage, transfer the vials to liquid nitrogen tank.

Thawing

1. Aseptically dispense 10 mL of IS BAC medium into a culture flask and pre-equilibrate to 28°C in air.

2. Thaw frozen cryovial of insect cells quickly in a 25°C water bath until just thawed.

3. Transfer contents of cryovial into the pre-equilibrated culture flask, and incubate at 28°C for 1-2 hours until cells settle into a monolayer. Gently aspirate medium (to remove the cryoprotectant) without disturbing cell monolayer and replace with fresh pre-warmed (28°C) IS BAC.

4. Monitor cell morphology and density daily, and when cell monolayer is about 90% confluent (~2 days post thawing), dislodge the cells from the flask by tapping or sloughing. To scale up to a shaker flask, transfer the entire flask contents into a shaker flask containing 20 mL of pre-equilibrated fresh IS BAC (30 mL total volume with cells). Incubate at 28°C with shaking at 130 rpm in air (no CO_2). If culturing in T-flask is preferred, subculture 4mL of the initial thawed culture into 8 mL of pre-equilibrated fresh IS BAC (12 mL total volume with cells).

5. Check cells daily for revival and determine density and viability every 2-3 days. Incubate cells until the viable cell density reaches 2-3 X 10^6 cells/mL.

6. Subculture into fresh IS BAC at a starting density of 3 X 10^5 cells/mL and maintain as previously described.

QUALITY ASSURANCE

IS BAC is tested as specified on the Certificate of Analysis.

PRODUCT AVAILABILITY

IS BAC is available in 1L bottles. Larger packaging and powder configurations can be arranged.