Allele Sf9 Insect Cells

The Sf9 cell line was derived from pupal ovarian tissue of the Fall armyworm Spodoptera frugiperda. The Sf9 cell line is highly susceptible to infection with Autographa california nuclear polyhedrosis virus (AcNPV baculovirus), and can be used with all baculovirus expression vectors. Sf9 cells are commonly used to isolate and propagate recombinant baculoviral stocks and to produce recombinant proteins.

Box 1 | Basic Info

Cat. No. ABP-CEL-10006
Contents
>1 x 10^7 viable Sf9 cells in 1 ml TNM-FH medium containing 7.5% DMSO. (Frozen)
Storage
Store at -80°C for up to one month. For longterm stability, store in liquid nitrogen.

Cat. No. ABP-CEL-10002
Contents
>1 x 10^7 Sf9 cells in 70 ml of TNM-FH Insect Culture Medium. *(Culture)*
Storage
Cells are shipped at room temperature. **Process cells immediately upon receipt.**

Protocols

ABP-CEL-10006
Frozen Cells

All procedures should be carried out under strict aseptic conditions in a sterile hood.

1. Thaw Sf9 cells by placing cryovial in a 27°C waterbath with vigorous agitation (do not immerse cap).

2. Spray cryovial with 70% ethanol, wipe it dry. Transfer contents to 15 ml sterile tube containing 10 ml of insect medium (TNM-FH, Cat.# ABP-MED-10001 for Sf9 cells).

3. Spin down the cells at 1,200 rpm for 3 minutes. Discard the supernatant and re-suspend the cell pellet in 10 ml of fresh insect cell medium. Repeat this cell washing once again to remove DMSO completely.

4. Discard the supernatant and re-suspend the cell pellet in 20 ml of fresh insect cell medium. Transfer cell suspension to sterile 10 cm tissue culture dish or T75 tissue culture flask. Incubate at 27°C. No CO_2 required.

5. After 48 hours, count cells, determine their viability and subculture them.

6. Split the cultures 1:3 when cells become 90% confluent.

7. Displace cells from the flask's surface by rapping the flask sharply against your hand 3 or 4 times (>75% of the cells should be detached from the surface of the flask).

8. Transfer the cell suspension into a microcentrifuge tube. Determine viability using the trypan blue exclusion method and determine cell density electronically using a Coulter Counter or manually using a hemocytometer chamber.

9. Seed appropriate amount of cells in appropriate vessels (4 - 7 x 10^6 cells to a T75 tissue culture flask or 6-9 x 10^6 cells to a T150 tissue culture flask). For suspension culture, start culture in a tissue culture flask for several passages, then transferring the cells to a suspension flask of suitable size at a minimum density of 1 x 10^6 cells/ml. Suspension culture should be diluted to 5 x 10^5 cells/ml when cells reach a density of 2 x 10^6 cells/ml. If suspension cultures are grown in shaker flasks, addition of 0.1%`plutonic F68 is necessary to prevent cell shear.

ABP-CEL-10002
Cell Culture

All procedures should be carried out under strict aseptic conditions in a sterile hood.

Cells are propagated in TNM-FH Medium (Cat. No. ABP-MED-10001)

1. Transfer a small aliquot of the cell suspension to a microcentrifuge tube.

2. Determine viability using the trypan blue exclusion method.

3. Determine cell density electronically using a Coulter Counter or manually using a hemocytometer chamber.

4. Transfer the 4 - 7 x 10^6 cells to a T75 tissue culture flask or 6-9 x 10^6 cells to a T150 tissue culture flask and allow the cells to attach for 30 minutes at room temperature.

5. Replace the medium with fresh TNM-FH medium: 15 ml for a T75 flask or 30 ml for a T150 flask.

6. Propagate at 27°C. Cells should begin dividing within 2 days.

7. Split the cultures 1:3 when cells become 90% confluent.

8. Displace cells from the flask's surface by rapping the flask sharply against your hand 3 or 4 times (>75% of the cells should be detached from the surface of the flask).

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8. Displace cells from the flask's surface by rapping the flask sharply against your hand 3 or 4 times (>75% of the cells should be detached from the surface of the flask).

9. Transfer the cell suspension into a microcentrifuge tube and determine cell count and viability (repeat Steps 1-3).

10. Seed appropriate amount of cells in appropriate vessels (4 – 7 x 10^6 cells to a T75 tissue culture flask or 6-9 x 10^6 cells to a T150 tissue culture flask). For suspension culture, start culture in a tissue culture flask for several passages, then transferring the cells to a suspension flask of suitable size at a minimum density of 1 x 10^6 cells/ml. Suspension culture should be diluted to 5 x 10^5 cells/ml when cells reach a density of 2 x 10^6 cells/ml. If suspension cultures are grown in shaker flasks, addition of 0.1% plutonic F68 is necessary to prevent cell shear.

References

1. *Smith, G.E. et al. Proc. Nat. Acad. Sci., USA (1985) 82: 8404-8;*

2. *Vaughn, J.L. et al. (1977) In Vitro 13: 213-217.*

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