VE-BEVS Insect Cell Lines:
VE-CL-01 Product No. 10010
VE-CL-02 Product No. 10020
VE-CL-03 Product No. 10030

VE-BEVS Cell Line United States Patent 7,842,493

VE-BEVS Cell Lines FAQs (Frequently Asked Questions)

1. What cell line were the VE-cell lines derived from?
2. Do I need to keep the VE-cell lines under antibiotic selection to prevent loss of expression of the vankyrin protein?
3. What is the average size of the VE-cells?
4. What media are VE-cells frozen in?
5. How many passages may I maintain my cells?
6. How do I start my VE-cell lines?
7. How do I freeze my cells down?
8. Which VE-cell line should I purchase?

1. What cell line were the VE-cell lines derived from?

The VE-cell lines are engineered from Sf9 cells that were originally derived from the ovarian tissue of the fall armyworm (*Spodoptera frugiperda*).

2. Do I need to keep the VE-cell lines under antibiotic selection to prevent loss of expression of the vankyrin protein?

No, the VE-cell lines are stably transformed cell lines and antibiotic treatment is not needed to maintain vankyrin protein expression.

3. What is the average size of the VE-cells?

While derived from Sf9 cells the VE-cells are larger in size than the parental Sf9 cells. VE-cells are approximately 21 microns in diameter.

4. What media are VE-cells frozen in?

Cells were frozen in 50% fresh Sf-900 II serum free medium, 50% conditioned Sf-900 II serum free medium and Dimethyl Sulfoxide (DMSO) to a final concentration of 7.5%

4. What media may I grow my VE-cells in?

The VE-cells are grown in Sf900 II media (Invitrogen™). However, the cells may be adapted to TNM-FH media supplemented with 10% FBS or in Expression Systems' ESF 921 media.

5. How many passages may I maintain my cells?

We recommend starting new cells after they have been through 30 passages. A passage is considered anytime you add media to dilute your cells.

6. How do I start my VE-cell lines?

Thawing Cells:

Thaw frozen cells rapidly in a 37°C water bath. Decontaminate the outside of the vial with 70% ethanol before transferring the cells into one T-25 cm² flask. WE RECOMMEND STARTING THE CELLS IN ADHERENT CULTURE AND THEN ADAPTING TO SHAKER CULTURE AFTER 2 PASSAGES.

Adherent Culture: Add 1 ml of Sf-900 II serum-free medium (SFM) into the cryovial containing thawed cells. Gently resuspend the cells. Remove the cells from the cryovial and transfer into one T25 flask containing 5 mL of medium. Transfer flasks to a 27^0C incubator and allow the cells to attach for 45-60 minutes before replacing the medium with 5 mL fresh Sf-900 II SFM. Subculture cells when they have reached a density of >80% confluency. Release cells from the flask's surface by tapping the flask sharply against your palm until > 75% of the cells have detached and transfer 2 mL cells into a new T25 flask containing 3 mL of medium.

Suspension Culture: To start a suspension culture, release the cells from two T25 monolayer cultures and transfer the entire volume from one flask and 3 mL from the second flask (a total volume of 8 ml) into a 125 mL shaker flask containing12 mL of fresh Sf-900 II SFM. Use the remaining 2 mL of cells to continue the cell line as adherent culture in a T-25 flask.

Incubate Erlenmeyer flask in a 27°C incubator on an orbital shaker platform rotating at 100-110 rpm. Loosen caps of flasks to allow proper oxygenation/aeration. Allow the cells to grow for 3-4 days. Count the cells from the starter flask and transfer the volume of cells necessary to reach a seeding density of $1x10^6$ cells/mL in 50 mL of Sf-900 II SFM in a 125 mL shaker flask. Once a suspension culture has been established and a cell density of $5-8x10^6$ viable cells/mL has been reached VE cells are routinely diluted to a cell density of $0.8-1x10^6$ viable cells/mL with Sf-900 II SFM.

7. How do I freeze my cells down?

Freeze cells at a density of $>2x10^7$ viable cells/mL in a freezing medium composed of 50% fresh Sf-900 II serum free medium (SFM), 50% conditioned Sf-900 II serum free medium and Dimethyl Sulfoxide (DMSO) to a final concentration of 7.5% (optional freezing media: fresh Sf-900 II SFM (Invitrogen™), 10% heat-inactivated FBS and DMSO to a final concentration of 7.5%). Centrifuge cells at 100g at 4°C for 5-10 minutes, remove the supernatant and re-suspend the pellet in an appropriate volume of chilled freezing medium to reach a density of $>2x10^7$ viable cells/mL. Transfer suspension into a cryovial. Place cells in a styrofoam container and place at -20°C for one hour, then transfer the styrofoam container with cells to -80°C overnight before transferring the cells to liquid nitrogen (vapor phase). Frozen cells remain viable if properly stored in liquid nitrogen.

8. Which VE-cell line should I purchase?

VE-Sf9-01 (Product #10010) displays the most prolonged enhanced protein expression (up to 7 days post-infection) when using conventional BEVS viruses. We recommend **VE-Sf9-01** for laboratories working with **highly stable intracellular and secreted proteins** as the pronounced enhancement of viability in these cells allows for prolonged expression and accumulation of recombinant protein over time.

VE-Sf9-02 (Product # 10020) displays a sharp peak of enhanced protein expression at day 3 and day 4 post-infection. We recommend **VE-Sf9-02** for laboratories working with **highly unstable or toxic proteins** as enhanced protein expression occurs at a very high level and over a very short time interval in these cells.

VE-Sf9-03 (Product # 10030) displays an intermediate phenotype to **VE-Sf9-01** and **VE-Sf9-02** showing significantly enhanced protein expression and moderately enhanced longevity. Due to its intermediate expression properties, we recommend **VE-Sf9-03** for laboratories working with proteins of **unknown toxicity and stability** or for **general enhancement of most recombinant proteins** expressed in conventional recombinant BEVS viruses.