Vankyrin-Enhanced Insect Cell Line

Product No.: 10010; 10020; 10030

Content
Product No. 10010 (previously VE-CL-01), 10020 (previously VE-CL-02) and 10030 (previously VE-CL-03) contain >$1x10^7$ cells in 50% fresh Sf-900 II serum free medium (Invitrogen™), 50% conditioned Sf-900 II serum free medium and Dimethyl Sulfoxide (DMSO) to a final concentration of 7.5%.

- 10010 has a cell doubling time of 37.6 hrs in Sf-900 II SFM and an average cell size of 21.6 µm.
- 10020 has a cell doubling time of 29.7 hrs in Sf-900 II SFM and an average cell size of 21.3 µm.
- 10030 has a cell doubling time of 43.6 hrs in Sf-900 II SFM and an average cell size of 21.7 µm.

Shipping and Storage
Cells are shipped on dry ice and are supplied in a cryogenic vial containing >$1x10^7$cells/mL. Cells were frozen in a freezing medium composed of 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%. Store cells in liquid nitrogen (vapor phase).
Live cells can be shipped upon request.

Product Qualification
To qualify for sales cells must be in logarithmic growth with 98% viability and less than 20 passages before they are frozen. Cells have been shown to recover as healthy logarithmically growing cells within 3 days after thawing.

Caution
DMSO is a hazardous material and caution has to be taken when handling this substance.

Cell Maintenance and Handling of Cells

Medium Requirement
Use of Sf-900 II SFM (Invitrogen) medium is recommended but cells also grow well in TNM-FH Insect Cell Culture Medium supplemented with 10% heat-inactivated FBS. **NOTE:** The addition of 400µg/mL neomycin should not be done until the first passage after thawing. Neomycin is optional for cell maintenance. **We recommend starting the cells in adherent culture and then adapting to shaker culture after 2 passages.**

Thawing Cells
Thaw frozen cells rapidly in a 37^0C water bath. Decontaminate the outside of the vial with 70% ethanol before transferring the 1 mL cell suspension into **two T-25 cm^2** flasks.

Adherent Culture: Put 0.5 mL of thawed cells into 5 mL of medium and transfer flask to a 27^0C incubator and allow the cells to attach for 30-45 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 3-4 times (> 75% of the cells should be 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 27^0C incubator on an orbital shaker platform rotating at 100-110 rpm. Loosen caps of flasks to allow proper oxygenation/aeration. When a cell density of $2x10^6$ viable cells/mL has been reached, cells should be seeded to a density of $1x10^6$ cells in 50 mL of Sf-900 II SFM in a 125 mL shaker flask. Once a suspension culture has been established VE cells are routinely diluted to a cell density of $7\text{-}8x10^5$ viable cells/mL with Sf-900 II SFM.

VE cells have an average diameter of approximately 21 µm which is bigger than Sf9 cells. In addition, VE cells grow slower than Sf9 cells.

Freezing Cells
Freeze cells at a density of $\geq 2x10^7$ viable cells/mL in a freezing medium composed of fresh Sf-900 II serum free medium (Invitrogen), 10% heat-inactivated FBS and DMSO to a final concentration of 7.5%. (**Optional freezing media:** 50% conditioned Sf-900 II media: 50% fresh Sf-900 II and DMSO to a final concentration of 7.5%**).** Centrifuge cells at 100g at 4°C for 5-10 minutes, remove the supernatant and resuspend the pellet in an appropriate volume of chilled freezing medium to reach a density of $\geq 2x10^7$ 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.

Overview of Vankyrin-Enhanced (VE) Insect Cell Line

Vankyrin-Enhanced Insect Cells (VE cells) are transgenic insect Sf9 cells that have been engineered to stably express the *Campoletis sonorensis* ichnovirus P-vank-1 protein (Fath-Goodin et al., 2006; Kroemer and Webb, 2006). Sf9 cells originated from the IPLBSF-21 cell line, derived from the pupal ovarian tissue of the fall army worm, *Spodoptera frugiperda* (Vaughn et al., 1977).

The stably transformed VE insect cell line was obtained by transfecting Sf9 cells with ParaTechs' proprietary transformation

vector harboring the P-vank-1 gene and the neomycin resistance gene. Neomycin was then used to select for stable cell lines. The expression of the P-vank-1 transcript was confirmed by RT-PCR.

The presence of the P-vank-1 protein leads to prolonged longevity and increased recombinant protein production of baculovirus infected VE cells compared to regular Sf9 cells. This cell line has been developed for enhanced recombinant protein production using the baculovirus expression vector system (BEVS).

- Modified insect Sf9 cells stably expressing a *Campoletis sonorensis* ichnovirus *vankyrin* gene
- Use of neomycin for selection of stable lines
- Prolonged longevity of cells after infection with a BEVS
- Up to 14-fold increase in protein yield as compared to regular Sf9 cells. Further enhancement (up to more than 20-fold) can be obtained by using modified cells in combination with the VE-BEVS transfer vector
- Compatible with all conventional BEVS
- Essentially no additional work or adaptation required
- **Expression of recombinant protein may need to be optimized**

This product is intended for research purposes only

CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

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Product Use Limitation

The VE-Cell Line ("Product") was developed in collaboration by scientists at ParaTechs and the University of Kentucky Lexington for expression of recombinant proteins. One or more patents or patent applications owned by the University of Kentucky Lexington cover components of the Product.

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Inquiries for commercial use should be directed to sbass@paratechs.com.

Patent information:
United States Patent Application 20060134743

References

Fath-Goodin, A., Kroemer, J.A., Martin, S.B., Reeves, K., and Webb, B.A. (2006). Polydnavirus genes that enhance the Baculovirus Expression Vector System. Advances in Virus Research, vol. 68, pp. 75-90.

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Vaughn, J.L., Goodwin, R.H., Tompkins, G.J. and McCawley, P. (1977). The establishment of two cell lines from the insect *Spodoptera fugiperda* (Lepidoptera: Noctuidae).

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ParaTechs Corporation
1122 Oak Hill Drive
Lexington, KY 40505

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