Vankyrin-Enhanced Insect Cell Line

Product No.: 10010; 10020; 10030

Content

Product No. 10010, 10020 and 10030 contain $\geq 1x10^7$ cells in 50% fresh Sf-900 II serum free medium (SFM; Gibco™), 50% conditioned Sf-900 II SFM and Dimethyl Sulfoxide (DMSO) to a final concentration of 7.5%.

Shipping and Storage

Cells are shipped on dry ice and are supplied in a cryogenic vial containing $\geq 1x10^7$cells/mL. Cells were frozen in a freezing medium composed of 50% fresh Sf-900 II SFM, 50% conditioned Sf-900 II SFM 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.

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

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.

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Cell Maintenance and Handling of Cells

Medium Requirement: Use of Sf-900 II SFM (Gibco) 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 G418 is not essential and might interfere with trypan blue staining of cells.
WE RECOMMEND STARTING THE CELLS IN ADHERENT CULTURE AND THEN ADAPTING TO SHAKER CULTURE ONCE ESTABLISHED.
IF THE CELLS DO NOT APPEAR TO BE VIABLE, DO NOT ATTEMPT TO REVIVE THE SECOND VIAL OF CELLS PROVIDED UNTIL YOU HAVE CONTACTED PARATECHS CORP FOR ADVICE.

Thawing Cells: Thaw frozen cells rapidly. Decontaminate the outside of the vial with 70% ethanol before transferring the 1 mL cell suspension into **one T-25 cm²** flask.

Adherent Culture: Add 1 ml of Sf-900 II SFM into the cryovial containing thawed cells. Gently resuspend the cells. Remove the cells from the cryovial and add to a T25 flask containing 4 mL of medium. Transfer flask to a 27°C 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 each of two new T25 flask containing 3 mL of medium. When these flasks have reached a density of >80% confluency, pass as above to generate a total of four flasks of cells. Let grow to >80% confluency and proceed to **Suspension Culture**.

Suspension Culture: To start a suspension culture, release the cells from three T25 monolayer cultures and transfer the entire volume (a total volume of 15 ml) into a 125 mL shaker flask containing 15 mL of fresh Sf-900 II SFM. It is recommended that the cell line also be continued as adherent culture in a T25 flask as a back-up source of cells.
Incubate shaker 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 $5\text{-}8x10^6$ viable cells/mL has been reached VE cells are routinely diluted to a cell density of $0.8\text{-}1x10^6$ 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.

- VE-CL-01 Cells (Product No. 10010) have a cell doubling time of 37.6 hrs in Sf-900 II SFM and an average cell size of 21.6 µm.
- VE-CL-02 Cells (Product No. 10020) have a cell doubling time of 29.7 hrs in Sf-900 II SFM and an average cell size of 21.3 µm.
- VE-CL-03 Cells (Product No. 10030) have a cell doubling time of 43.6 hrs in Sf-900 II SFM and an average cell size of 21.7 µm.

Freezing Cells: Freeze cells at a density of $\geq 2x10^7$ viable cells/mL in a freezing medium composed of fresh Sf-900 II SFM, 10% heat-inactivated FBS and DMSO to a final concentration of 7.5%. (**Optional freezing media:** 50% conditioned Sf-900 II SFM: 50% fresh Sf-900 II and DMSO to a final concentration of 7.5%). Centrifuge cells at 100g at 4ºC 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 1ml aliquots into a cryovial. Place cells in a styrofoam container and place at -20ºC for one hour, then transfer the styrofoam container with cells to –80ºC 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 (Steele et al., 2017; 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. Geneticin G418 Sulfate 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 standard Sf9 cells. This cell line has been developed for enhanced recombinant protein production using the baculovirus expression vector system (BEVS). The key features are:

- Modified insect Sf9 cells stably expressing a *Campoletis sonorensis* ichnovirus *vankyrin* gene
- Use of G418 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 standard Sf9 cells. Further enhancement (up to more than 20-fold) can be obtained by using modified cells in combination with a VE-BEVS transfer vector (Product No. 20010, 20020 or 20030)
- Compatible with all conventional BEVS
- Essentially no additional work or adaptation required
- **Expression of recombinant protein may need to be optimized by testing different time points and MOIs.**

This product is intended for research purposes only.

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

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

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Product Use Limitation:
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References:
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Vaughn JL, Goodwin RH, Tompkins GJ and McCawley P (1977). The establishment of two cell lines from the insect *Spodoptera fugiperda* (Lepidoptera: Noctuidae).

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