SAFC Biosciences™
Accelerate Success™

Product Information

EX-CELL™ VPRO Serum-Free Medium for Retinoblast Cells

with L-glutamine, without sodium bicarbonate

CATALOG NO. 24561C

Description

EX-CELL™ VPRO is an animal-protein free, serum-free dry powder medium developed for the long-term growth of PER.C6® and related cell lines. The cells can be subcultured directly into EX-CELL™ VPRO from other serum-free media with little or no adaptation. PER.C6® cells can be grown as suspension cultures either in shaker flasks or roller bottles, with roller bottles being the preferred culture system. Suspension cultures can be maintained, without refeeding for approximately 10 days and can be carried for more than 20 passages with no loss of viability.

Catalog No. 24561C replaces Catalog No. 24560 and includes an alternate source of soy hydrolysate to that found in the original EX-CELL™ VPRO formulation. The new formulation also contains a synthetic D-galactose, which replaces bovine milk-derived D-galactose. The alternate hydrolysate offers more consistent performance and improved filtration characteristics, which will improve the overall performance and consistency of EX-CELL™ VPRO. In both cases, comparability testing utilizing the previous components and the replacement components demonstrated comparable growth-promoting characteristics.

Formulation

The formula for EX-CELL™ VPRO is proprietary to SAFC Biosciences. For additional information please call our Technical Services department.

Precautions

Use aseptic technique when handling or supplementing this medium. This product is for research or for further manufacturing use. THIS PRODUCT IS NOT INTENDED FOR HUMAN OR THERAPEUTIC USE.

Storage

Store dry powder medium at 2 to 8 C. Store hydrated medium at 2 to 8 C, protected from light. Do not use after the expiration date.

Indications of Deterioration

Medium should be free flowing. Do not use if medium is caked. Hydrated medium should be clear and free of particulates and flocculent material. Do not use if liquid medium is cloudy or contains precipitate. Other evidence of deterioration may include color change, pH shift or degradation of physical or performance characteristics.

Preparation Instructions

Dry powder medium is vacuum dried, where appropriate, during the particle reduction process and packaged in a humidity-controlled environment. This treatment ensures maximum dehydration and product stability. The end product is extremely hygroscopic and must be protected from atmospheric moisture. We recommend that the entire contents of each package be used immediately after opening. Preparing concentrated solutions is not recommended because of the low solubility coefficients of some amino acids and the tendency of some salts to form insoluble complexes.

EX-CELL™ VPRO is formulated with L-glutamine and without sodium bicarbonate.

1. Measure 80 - 90% of final required volume of cell culture grade water (Catalog No. 59900C) into an appropriate size mixing vessel. Water temperature should be 20 to 30 C.
2. Slowly add 21.22 g/L of EX-CELL™ VPRO dry powder medium, allowing mixing time between additions. Rinse the package with a small amount of cell culture grade water to remove traces of powder and add to the solution.

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3. Adjust the pH to 5.0 with HCl 1N, mix to equilibrate and adjust the pH to 6.8 with NaOH 1N (Catalog No. 59223C).
4. Mix for 30 minutes before adding 1.8 g/L of sodium bicarbonate (Catalog No. 90421C) or 24 mL/L of sodium bicarbonate solution 7.5% (Catalog No. 59221C). Mix until fully dissolved.
5. While mixing the solution, adjust the pH to 6.9 - 7.1 using NaOH 1N or HCl 1N. The pH of this medium usually rises 0.1 - 0.2 units during filtration. For most applications, the optimal pH of the filtered medium is 7.0 - 7.4.
6. Add cell culture grade water to the solution to bring it to final volume. Continue to mix for at least 60 minutes. To avoid fluctuation in pH, keep the vessel closed until the medium is filtered.
7. To sterilize the medium, first use a low protein-binding 0.45 µm pre-filter, followed by a sterilizing low protein-binding membrane filter with a pore size of 0.22 µm. To minimize CO_2 loss, a peristaltic pump or an inert gas, such as nitrogen, can be used to provide positive pressure at 2 - 15 psi. Do not use CO_2 gas.

   NOTE: Other supplements, such as antibiotics or L-glutamine, can be added to the sterilized medium using aseptic technique. SAFC Biosciences recommends the supplementation of 10 - 25 mM HEPES buffer in applications outside of a pH-controlled environment (such as stationary T-flasks, roller bottles and spinner flasks) by supplementing with 10 - 25 mL/L of HEPES Solution 1M (Catalog No. 59205C). Storage conditions and shelf life of the supplemented product may be affected by the nature of the supplements.
8. Dispense medium into sterile containers using aseptic technique. Store liquid medium protected from light at 2 to 8 C.

Methods for Use

Adaptation

PER.C6® cells that have been grown in a serum-free medium can be readily grown in EX-CELL™ VPRO with little or no adaptation. Adaptation of PER.C6® cells in EX-CELL™ VPRO requires healthy, viable cultures in mid-logarithmic growth phase. PER.C6® cells can be subcultured directly into EX-CELL™ VPRO without any loss in viability.

1. Subculture the cells from serum-free medium directly into EX-CELL™ VPRO at a density of 2.5-3 x 10^5 cells/mL.
2. Allow the cells to adapt to EX-CELL™ VPRO Medium for an additional 4 - 6 passages. Cells are considered fully adapted to EX-CELL™ VPRO when growth rates return to normal and viabilities are above 95%.
3. Continue to subculture cells in EX-CELL™ VPRO at a density of at least 2.5 x 10^5 cells/mL using roller bottles or shaker flasks.

Culture Techniques

PER.C6® cells are normally grown at 37 ± 1 C and 10% CO_2. Allow the medium to warm to room temperature prior to use. Once fully adapted, the cells should be passed at a seeding density of 2.5-3 x 10^5 cells/mL in roller bottles. Seed 100 mL cell cultures in 490 cm^2 roller bottles, with a roller speed of 1 rpm.

When passing the cells, medium carry over should not exceed 25% of the final volume. If carry over exceeds 25%, centrifugation is recommended. Cells propagated in serum-free media are extremely fragile. For successful results, care must be taken when subculturing cells. Standard techniques of centrifugation must be modified to include low-speed centrifugation to prevent damage to cells that have been propagated in serum-free medium.

Cryopreservation

Freezing:

Cells can be frozen in EX-CELL™ VPRO without the reintroduction of serum.

1. Choose cultures in logarithmic growth with viabilities above 90%.
2. Prepare a freezing medium consisting of 45% cold EX-CELL™ VPRO, 45% spent medium and 10% dimethyl sulfoxide (DMSO).
3. Centrifuge the cells at 200 *g* for 5 minutes. Remove the supernatant.
4. Resuspend the cells in the freezing medium at 5 x 10^6 to 1 x 10^7 cells/mL.
5. Rapidly transfer 1 - 2 mL of this suspension to sterile cryovials.
6. Place the vials at -20 C for 3 - 4 hours, then transfer to -70 C for 16 - 24 hours.
7. For long-term storage, transfer the vials to liquid nitrogen vapor.

Thawing:

1. Rapidly thaw a vial of frozen cells in a 37 C water bath.
2. Transfer the cells aseptically to a centrifuge tube containing 10 mL of chilled EX-CELL™ VPRO medium.
3. Using low-speed centrifugation, pellet the cell suspension at 200 *g* for 5 minutes and carefully decant the supernatant without disturbing the cell pellet.
4. Resuspend the cells in 5 mL of EX-CELL™ VPRO medium.
5. Count the cells for viability and transfer to a sterile tissue culture flask at a seeding density of 2.5-3 x 10^5 cells/mL.
6. Pass the cells using standard cell culture techniques and transfer to roller bottles as cell densities increase.

Characteristics

Appearance
White free-flowing powder

Bioburden
Refer to Certificate of Analysis

Endotoxin
Refer to Certificate of Analysis

Osmolality (as supplied)
Refer to Certificate of Analysis

pH (as supplied)
Refer to Certificate of Analysis

Warranty, Limitation of Remedies

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Additional Terms and Conditions are contained in the product Catalog, a copy of which is available upon request.

Issued September 2006 P24561
0604 0805 0905 0406

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