

Product Information

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

without L-glutamine

CATALOG NO. 14561C

Description

EX-CELL™ VPRO is an animal-protein free, serum-free liquid medium developed for the long-term growth of Human Embryonic Retinoblast cells (PER.C6® and related cell lines). PER.C6® cells can be subcultured directly into EX-CELL™ VPRO from 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. 14561C replaces Catalog No. 14560 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 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 liquid medium at 2 to 8 C, protected from light. Do not use after the expiration date.

Indications of Deterioration

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

Preparation Instructions

EX-CELL™ VPRO is formulated with sodium bicarbonate and without L-glutamine. Prior to use, this medium should be supplemented with 6 mM L-glutamine by adding 30 mL/L of a 200 mM solution (Catalog No. 59202C). SAFC Biosciences recommends L-glutamine supplementation of the working volume only. SAFC Biosciences also 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). Supplements, such as antibiotics, can be added to the sterilized medium using aseptic technique. Storage conditions and shelf life of the product may be affected by the nature of the supplement.

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.

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1. Subculture the cells from serum-free medium directly into EX-CELL™ VPRO at a density of 2.5-3 x 10⁵ cells/mL.
2. Allow the cells to adapt to EX-CELL™ VPRO 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 10⁵ cells/mL using roller bottles or shaker flasks.

Culture Techniques

PER.C6® cells are normally grown at 37 ± 1 C and 10% CO₂. 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 10⁵ cells/mL in roller bottles. Seed 100 mL cell cultures in 490 cm² 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 culture in logarithmic growth with viability above 90%.
2. Prepare a freezing medium consisting of 45% cold EX-CELL™ VPRO medium, 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 10⁶ to 1 x 10⁷ 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 10⁵ cells/mL.
6. Pass the cells using standard cell culture techniques and transfer to roller bottles as cell densities increase.

Characteristics

Appearance

Clear yellow solution

Endotoxin

Refer to Certificate of Analysis

Osmolality (as supplied)

260 - 300 mOsm/kg H₂O

pH (as supplied)

7.0 - 7.4

Sterility

No microbial growth detected

Warranty, Limitation of Remedies

SAFC Biosciences warrants to the purchaser for a period of one year from date of delivery that this product conforms to its specifications. Other terms and conditions of this warranty are contained in SAFC Biosciences' written warranty, a copy of which is available upon request. ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING THE IMPLIED WARRANTY OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE, ARE EXCLUDED. In no case will SAFC Biosciences be liable for any special, incidental, or consequential damages arising out of this product or the use of this product by the customer or any third party based upon breach of warranty, breach of contract, negligence, strict tort, or any other legal theory. SAFC Biosciences expressly disclaims any warranty against claims by any third party by way of infringement or the like. THIS PRODUCT IS INTENDED FOR PURPOSES DESCRIBED ONLY AND IS NOT INTENDED FOR ANY HUMAN OR THERAPEUTIC USE.

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

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Issued September 2006 P14561
0604 0805 0905 0306

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