

Product Information

EX-CELL™ EBx® GRO-I Serum-Free Medium for Embryonic Stem Cells

without L-glutamine

CATALOG NO. 14530C

Description

EX-CELL™ EBx® GRO-I is an animal-component free, serum-free liquid medium developed for the growth of EB66® cells. The EB66® cell line is proprietary to Vivalis (Saint-Herblain, France). The EB66® cell line is a fully characterized duck cell line utilized in cell-based vaccine manufacturing and for the production of recombinant viral vectors and therapeutic recombinant proteins.

Formulation

The formulation for EX-CELL™ EBx® GRO-I 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 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 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

EX-CELL™ EBx® GRO-I is formulated without L-glutamine. Prior to use, this medium should be supplemented with 2.5 mM L-glutamine by adding 12.5 mL/L of a 200 mM solution (Catalog No. 59202C). SAFC Biosciences recommends L-glutamine supplementation of the working volume only. Supplements, such as antibiotics, can be added to the sterilized medium using aseptic technique. The addition of a surfactant (such as Pluronic® F-68) is not required. Storage conditions and shelf life of the product may be affected by the nature of the supplement.

Methods for Use

Adaptation

EB66® cells can be readily cultured in EX-CELL™ EBx® GRO-I medium. Adaptation to EX-CELL™ EBx® GRO-I requires healthy, viable cultures in mid-logarithmic growth phase.

Culture Techniques

EB66® cells are normally grown at 37 ± 1 C and 7.5% CO₂. Pre-warm the medium to 37 C prior to use. Once fully adapted, the cells should be passed every 3 days at a seeding density of 2-3 x 10⁵ cells/mL in shaker flasks. Shaker speed should be 120 rpm.

When passing the cells, carryover should not exceed 25% of the final volume. If carryover exceeds 25%, centrifugation is recommended. Cells propagated in serum-free medium are extremely fragile. Standard techniques for centrifugation must be modified to include low-speed centrifugation (270 g) to prevent damage to cells that have been propagated in serum-free medium.

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Cryopreservation

Freezing:

EB66® cells grown in EX-CELL™ EBx® GRO-I medium have been successfully frozen in liquid nitrogen and recovered without the reintroduction of serum.

1. Choose cultures in logarithmic growth with viabilities above 90%.
2. Centrifuge the cells at 270 g for 5 minutes. Remove the supernatant.
3. Resuspend the cells in EX-CELL™ EBx® GRO-I at 2×10^7 cells/mL.
4. Add an equal volume of cooled (2 to 8 C) EX-CELL™ EBx® GRO-I medium with 20% DMSO.
5. Rapidly transfer 1 mL of this suspension to sterile cryovials. Maintain at 2 to 8 C until ready for freezing.
6. Place the vials at -70 C for 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 9 mL of pre-warmed EX-CELL™ EBx® GRO-I medium.
3. Using low-speed centrifugation, pellet the cell suspension at 270 g for 5 minutes and carefully decant the supernatant without disturbing the cell pellet.
4. Resuspend the cells in 10 mL of pre-warmed EX-CELL™ EBx® GRO-I medium.
5. Count the cells for viability and transfer to sterile 125 mL shaker flask. Monitor cells daily and subculture when cells reach $>2 \times 10^6$ cells/mL.

Characteristics

Appearance

Clear solution, free of precipitate

Endotoxin

<20.0 EU/mL

Osmolality (as supplied)

285 - 315 mOsm/kg H₂O

pH (as supplied)

7.0 - 7.4

Sterility

No microbial growth detected

Access to EB66® Cells

Please contact Vivalis at info@vivalis.com or +33(0)228-073710 for access to EB66® cells and to receive technical support on EB66® cells, culture and use.

Warranty, Limitation of Remedies

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