

Product Information

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

without L-glutamine, without sodium bicarbonate

CATALOG NO. 24530C

Description

EX-CELL™ EBx® GRO-I Serum-Free Medium for Embryonic Stem Cells is an animal-component free, serum-free dry powder medium formulated 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 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 and 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™ EBx® GRO-I is formulated without 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 19.06 g/L of EX-CELL™ EBx® GRO-I dry powder medium while stirring. Rinse the package with a small amount of cell culture grade water to remove traces of powder and add to the solution.
3. Mix for at least 30 minutes or until completely dissolved. Do not heat the medium.
4. Add 1.6 g/L of sodium bicarbonate (Catalog No. 90421C) or 21.3 mL/L of sodium bicarbonate solution 7.5%. 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 the 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 mixing for at least 30 minutes. To avoid fluctuation in pH, keep the vessel closed until the

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medium is filtered.

7. To sterilize the medium, sterile filter using a low protein-binding membrane filter with a pore size of 0.22 µm. For larger volumes, a low protein-binding 0.45 µm pre-filter is recommended. To minimize CO₂ 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 CO₂ gas.

NOTE: For applications requiring the use of L-glutamine, supplement with 2.5 mM L-glutamine by adding 12.5 mL/L of a 200 mM solution (Catalog No. 59202C) prior to use. SAFC Biosciences recommends L-glutamine supplementation of the working volume only. Other supplements, such as antibiotics, can be added to the sterilized medium using aseptic technique. 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

EB66® cells can be readily cultured in EX-CELL™ EBx® GRO-I medium. Adaptation to EX-CELL™ EBx® GRO-I medium 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 cultures are adapted, the cells should be passed every 3 days at a seeding density of 3 x 10⁵ cells/mL. Shaker speed should be 120 rpm.

When passing the cells, carry over should not exceed 25% of the final volume. If carryover exceeds 25%, centrifugation is recommended. 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.

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 medium at 2 x 10⁷ 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 pre-warmed EX-CELL™ EBx® GRO-I medium.
5. Count the cells for viability and transfer to a sterile 125 mL shaker flask. Monitor cells daily and subculture when cells reach >2 x 10⁶ cells/mL.

Characteristics

Appearance

Free-flowing powder

Endotoxin

Record EU/mL

Osmolality (without sodium bicarbonate)

Record mOsm/kg H₂O

pH (without sodium bicarbonate)

Record

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.

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