

Product Information

EX-CELL® EBx® PRO-II Serum-Free Medium

without L-glutamine, without sodium bicarbonate

CATALOG NO. 24533C

Description

EX-CELL® EBx® PRO-II is a chemically defined, animal-component free, serum-free, low-protein dry powder medium developed for Influenza virus propagation in EB66® embryonic stem cells in suspension culture. 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. As EX-CELL EBx PRO-II has been developed for viral propagation and is not designed to independently support cell growth, EX-CELL® EBx® GRO-I (Catalog No. 14530C) is required for routine culture and expansion of EB66 cell line followed by exchange or dilution with EXCELL EBx PRO-II for viral infection and amplification.

Formulation

The formulation for EX-CELL EBx PRO-II 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 PRO-II 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 12.44 g/L of EX-CELL EBx PRO-II 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 30 minutes or until completely dissolved. Do not heat the medium.
4. Add 3.07 g/L of sodium bicarbonate (Catalog No. 90421C) or 41.3 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 - 7.6.
6. Add cell culture grade water to the solution to bring it to final volume. Continue mixing for 30 - 60 minutes. To avoid fluctuation in pH, avoid prolonged mix times and keep the vessel closed until the 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 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: 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

Culture Techniques

EB66 cells are expanded to required pre-infection densities in EX-CELL EBx GRO-I (Catalog No. 24530C) growth medium. EB66 cells are normally grown at 37 ± 1 °C and 7.5% CO_2 in shaker flasks at 120 rpm.

Virus infection and production is induced with the addition of seed virus and 1.5 - 1.8 times (v/v) pre-warmed EX-CELL EBx PRO-II medium to growth medium. Standard infection cell densities are $>2 \times 10^6$ viable cells/mL (prior to dilution with production medium) at a multiplicity of infection (MOI) of 0.01 - 0.10.

Optimal conditions for virus amplification may vary depending on viral construct and process conditions. Recommended post-infection conditions are 33 to 37 °C and 7.5 % CO_2 in shaker flasks at 120 rpm.

Cryopreservation

EX-CELL EBx PRO-II is not recommended for cryopreservation of the EB66® cell line. Please refer to EX-CELL EBx GRO-I (Catalog No. 14530C) growth medium literature for recommended cryopreservation conditions.

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.

Warranty, Limitation of Remedies

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Issued March 2010 P24533
1208 1109