

Product Information

EX-CELL™ Vero Serum-Free Medium for Vero Cells

with L-glutamine, without sodium bicarbonate

CATALOG NO. 24585C

Description

EX-CELL™ Vero is a serum-free, animal-component free, low-protein dry powder medium developed for the long-term growth of African Green Monkey Kidney (Vero) cells. The cells, in an attachment culture, can be subcultured directly into EX-CELL™ Vero from serum-supplemented media with little or no adaptation. Cell densities and doubling times achieved are comparable to those achieved in a serum-supplemented culture.

Formulation

The formulation for EX-CELL™ Vero 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™ Vero 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 20.08 g/L of EX-CELL™ Vero 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.
3. Mix for at least 30 minutes until completely dissolved. Do not heat the medium.
4. Add 2.7 g/L of sodium bicarbonate (Catalog No. 90421C) or 36 mL/L of sodium bicarbonate solution 7.5% (Catalog No. 59221C). Mix until dissolved.
5. While mixing the solution, adjust the pH to 6.9 - 7.1 using NaOH 1N (Catalog No. 59223C). 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 and continue mixing for at least 60 minutes. To avoid fluctuations in pH, keep the vessel closed until the medium is filtered.

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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: 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 hydrated medium protected from light at 2 to 8 C.

Methods for Use

Adaptation

Vero cells that have been grown in a conventional serum-supplemented medium can be readily grown in EX-CELL™ Vero, with little or no adaptation. Adaptation to EX-CELL™ Vero requires healthy, viable cultures in mid-logarithmic growth phase. During adaptation, growth rates will usually be somewhat slower than normal expected rates.

1. Subculture the cells from serum-supplemented medium to EX-CELL™ Vero using standard trypsinization techniques when cultures reach 100% confluence.
2. Inactivate the trypsin with trypsin inhibitor from Glycine max (soybean) (STI) (0.1%) (Sigma-Aldrich Co., Product No. T6522) at a 1:4-5 relationship of trypsin to STI. Using low-speed centrifugation, pellet the cell suspension at 200 g for 5 minutes and carefully decant the supernatant without disturbing the cell pellet.
3. Resuspend the cells in EX-CELL™ Vero medium supplemented with 4 mM L-glutamine at a density of $2-4 \times 10^4$ cells/cm².
4. Cells are considered fully adapted to EX-CELL™ Vero when growth rates return to normal densities and viabilities are above 95%.
5. Continue to subculture the cells in EX-CELL™ Vero at a density of at least 2×10^4 cells/cm².

Culture Techniques

Vero cells are normally grown at 37 ± 1 C and 5% 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 at least 2×10^4 cells/cm².

Cells grown in medium without serum are extremely fragile and sensitive to the trypsin used to remove adherent cells from a substrate. For successful results, care must be taken when subculturing cells.

SAFC Biosciences recommends the use of a soybean trypsin inhibitor (0.1%), followed by centrifugation to remove the trypsin. Soybean trypsin inhibitor should be used with caution, as it is toxic to many cells. Cells may also be dislodged by NO-ZYME™ (Catalog No. 59226C), a non-enzymatic dissociating agent.

Cryopreservation

Freezing:

Cells can be frozen in EX-CELL™ Vero 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™ Vero medium, 45% spent medium and 10% dimethyl sulfoxide (DMSO).
3. Using trypsinization protocol as detailed previously, collect and centrifuge the cells at 200 g for 5 minutes. Remove the supernatant without disturbing the cell pellet.
4. Resuspend the cells in the freezing medium at 1×10^7 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 cold EX-CELL™ Vero medium.
3. Using low-speed centrifugation, pellet the cell suspension 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™ Vero medium.
5. Count the cells for viability and transfer to a sterile tissue culture flask at a seeding density of $2-4 \times 10^4$ cells/cm².
6. Pass the cells using standard cell culture techniques.

Characteristics

Appearance

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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