SAFC Biosciences™
Accelerate Success™

Product Information

EX-CELL™ 325 PF CHO Serum-Free Medium for CHO Cells, Protein-Free

without L-glutamine, without sodium bicarbonate

CATALOG NO. 24340C

Description

EX-CELL™ 325 PF CHO is a protein-free, animal-component free dry powder medium which has been developed for the growth of Chinese Hamster Ovary (CHO) cells. Because it contains no large macromolecules, EX-CELL™ 325 PF CHO facilitates the isolation and purification of secreted proteins from the cells. CHO cells propagated in EX-CELL™ 302 serum-free medium (Catalog No. 14324C and 14326C) can be transferred directly into this protein-free medium without extensive weaning protocols.

This medium is supplied without L-glutamine and does not contain purines or pyrimidines to provide an appropriate medium for specialized CHO cell lines (i.e., Glutamine Synthetase, or GS System™, and DHFR$^-$ selection systems).

Catalog No. 24340C replaces Catalog No. 24335 and includes an alternate source of soy hydrolysate to that found in the original EX-CELL™ 325 PF CHO formulation. With more consistent performance and improved filtration characteristics, the alternate hydrolysate will improve the overall performance and consistency of EX-CELL™ 325 PF CHO. Comparability testing utilizing the previous soy hydrolysate and the replacement hydrolysate demonstrated comparable growth-promoting characteristics.

Formulation

The formulation for EX-CELL™ 325 PF CHO is proprietary to SAFC Biosciences. For additional information please call our Technical Services department.

Precautions

Use aseptic technique when handling or supplementing this medium after filtration. 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 or 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™ 325 PF CHO is formulated without L-glutamine and without sodium bicarbonate.

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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 21.04 g/L EX-CELL™ 325 PF CHO medium. Stir until completely dissolved. Rinse the package with a small amount of cell culture grade water to remove traces of powder and add to the solution
3. Mix 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% (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) 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. To avoid fluctuation in pH, 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 4 mM L-glutamine by adding 20 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

Adaptation

Most cells can be transferred directly from EX-CELL™ 302 serum-free medium into EX-CELL™ 325 PF CHO without adaptation. The following procedure is suggested for those cells that may require weaning (i.e., high protein, serum-free or serum-supplemented cultures):

1. Subculture actively growing cells by planting new cultures at 3-5 x 10^5 viable cells/mL into a mixture of the previous growth medium (serum-containing or serum-free "control" medium) and EX-CELL™ 325 PF CHO at a ratio of 3:1.
2. After 2 - 4 days, count cells and compare cell number and viability to the control cell system. Dilute cell culture with fresh EX-CELL™ 325 PF CHO to obtain the same seeding density outlined in Step 1.
3. If cell growth has been maintained at rates equivalent to those observed in the control cultures, continue the dilution process as described in Step 2.
4. Higher cell seeding densities may be required for the first few passages in EX-CELL™ 325 PF CHO alone. Once cells are fully adapted to EX-CELL™ 325 PF CHO, seeding densities can be adjusted to lower densities for initiating new cultures.

Culture Techniques

The transfer of cells from serum-supplemented medium directly into protein-free medium is not recommended. It is best to slowly reduce the concentration of protein in the culture environment and allow the cells to adjust. EX-CELL™ 302 serum-free media is compatible and well-suited for this interim period. Once successful growth of CHO cells has been established, they can be transferred into protein-free EX-CELL™ 325 PF CHO medium. Most CHO cell lines do not require a step-wise reduction from EX-CELL™ 302, and can be transferred directly into EX-CELL™ 325 PF CHO medium. A slight reduction in cell growth may be observed during the first few subcultures in this medium, but cells will rapidly adjust.

Once cultures are fully adapted, the cells should be passed every 3 - 4 days at a seeding density of at least 2-4 x 10^5 cells/mL. An optimal seeding density should be determined by the researcher for each application and cell type.

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 or protein-free medium are extremely fragile. Standard techniques for centrifugation must be modified to include low-speed centrifugation to prevent damage to cells that have been propagated in serum-free medium.

During adaptation, trypsin should be avoided if possible. If trypsin must be used, incubations should be carried out at 4 C, and exposure time should be minimal. SAFC Biosciences recommends the use of a soybean trypsin inhibitor (0.1%), or sedimentation by centrifugation to remove the trypsin. Soybean trypsin inhibitor should be used with caution, as it is toxic to some cell types. Cells may also be dislodged with NO-ZYME™ (Catalog No. 59226C), a non-enzymatic dissociating agent.

Cryopreservation

Freezing:

Cells can be frozen in EX-CELL™ 325 PF CHO 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™ 325 PF CHO medium, 45% conditioned 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 10^6 to 1 x 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 chilled EX-CELL™ 325 PF CHO 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™ 325 PF CHO medium.
5. Count the cells for viability and transfer to a sterile shaker flask at a seeding density of 2.5-3 x 10^5 cells/mL.
6. When cell densities reach 1-2 x 10^6 cells/mL, passage the cells using standard cell culture techniques.

Characteristics

Appearance
Off-white free-flowing powder

Bioburden
≤ 500 CFU/100 mL

Endotoxin
≤ 10.0 EU/mL

Osmolality (as supplied)
Refer to Certificate of Analysis

pH (as supplied)
Refer to Certificate of Analysis

Warranty, Limitation of Remedies

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

Issued September 2006 P24340
0104 0805 0905 0406

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