SAFC Biosciences™
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Product Information

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

without L-glutamine

CATALOG NO. 14340C

Description

EX-CELL™ 325 PF CHO is a protein-free, animal-component free liquid 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 media (Catalog No. 14324C/24324C or 14326C/24326C) 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 the GS System™, and DHFR$^-$ selection systems).

Catalog No. 14340C replaces Catalog No. 14335 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. 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 medium is cloudy or contains precipitates. Other evidence of deterioration may include color change, pH shift or degradation of physical or performance characteristics.

Preparation Instructions

EX-CELL™ 325 PF CHO is formulated with sodium bicarbonate and without L-glutamine. For applications requiring the use of L-glutamine, supplement with 4 mM L-glutamine prior to use by adding 20 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 medium using aseptic technique. Storage conditions and shelf life of the product may be affected by the nature of the supplements.

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.

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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
Clear orange-red solution

Endotoxin
≤ 10.0 EU/mL

Osmolality (as supplied)
316 - 356 mOsm/kg H_2O

pH (as supplied)
7.0 - 7.4

Sterility
No microbial growth detected

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