

# Technical Bulletin

## Frequently Asked Questions EX-CELL® CD CHO Serum-Free Medium

**CATALOG NO.: 14360C (w/o HT), 14361C (w/ HT), 24360C (w/o HT), 24361C (w/ HT)**

### What is the advantage of a chemically defined medium?

A chemically defined medium offers the advantages of lot-to-lot consistency and improved product performance. Furthermore, it allows for consistent control over protein production and facilitates downstream processing.

### When do I use EX-CELL CD CHO with HT vs. EX-CELL CD CHO without HT?

EX-CELL CD CHO (Catalog No. 14360 and Catalog No. 24360) is formulated without L-glutamine, hypoxanthine and thymidine (HT), making it an appropriate medium for systems that utilize the selective pressure of an HT-deficient or L-glutamine-deficient medium. For applications such as DHFR-, EX-CELL CD CHO without HT (Catalog No. 14360 and Catalog No. 24360) is the optimal choice.

EX-CELL CD CHO (Catalog No. 14361 and Catalog No. 24361) is formulated without L-glutamine, but does contain hypoxanthine and thymidine, making it suitable for applications that do not require the selective pressure of an HT-deficient medium. For CHO-K1 and GS System™ users, EX-CELL CD CHO that contains HT (Catalog No. 14361 and Catalog No. 24361) can improve overall growth performance and culture longevity.

### Why use EX-CELL CD CHO over other CHO serum-free media SAFC offers, such as EX-CELL 302 and EX-CELL 325?

While both EX-CELL 302 and EX-CELL 325 are excellent serum-free media for CHO cells, both contain a hydrolysate. A chemically defined media such as EX-CELL CD CHO does not contain a hydrolysate, offering the advantages of lot-to-lot consistency, as well as ease of downstream purification.

### What cell lines have been successfully cultured in EX-CELL CD CHO?

EX-CELL CD CHO has been shown to support the growth of numerous CHO clones, including CHO-K1, DXB11 and DG44.

### Does EX-CELL CD CHO support suspension or adherent cultures?

EX-CELL CD CHO is designed specifically for suspension-cultures only.

### What are the recommended cell culture conditions using EX-CELL CD CHO? What are the optimal parameters?

EX-CELL CD CHO	
Minimum Seeding Density	3 x 10 <sup>5</sup> cells/mL
Passage frequency	3 - 4 days
Supplements added at time of use	8 mM L-glutamine
Environment (temperature, CO <sub>2</sub> , agitation)	37 C (humidified incubator), 8 - 10% CO <sub>2</sub> and 120 - 130 rpm

### How do I adapt cells to this media? Is there the same long adaptation period experienced with other chemically defined CHO media?

CHO cells grown in a serum-containing media can be readily grown in EX-CELL CD CHO medium with little or no adaptation by using healthy, viable cultures in mid-logarithmic growth phase prior to adaptation. CHO cells grown as attachment or suspension cultures in a conventional serum-supplemented medium can be directly adapted to EX-CELL CD CHO without the extensive adaptation protocols and lengthy procedures required by other chemically defined CHO media. Please refer to the SAFC Product Information Sheet for each EX-CELL CD CHO product for more detailed information.

### How do I adapt from another chemically defined medium?

For most applications, CHO cells can be directly adapted to EX-CELL CD CHO using seeding densities of 4-5 x 10<sup>5</sup> cells/mL. However, some applications may require a gradual wean to EX-CELL CD CHO. Please contact Technical Services for additional information.

### Can I adapt from EX-CELL 302 or EX-CELL 325?

Yes, CHO cells readily adapt to EX-CELL CD CHO from both EX-CELL 302 and EX-CELL 325 using a direct adaptation-method and seeding densities of 4-5 x 10<sup>5</sup> cells/mL.

### Can EX-CELL CD CHO be used for transfection?

SAFC does not have data to adequately answer this question at this time.

#### **Does EX-CELL® CD CHO support clonal growth?**

EX-CELL CD CHO will support growth of CHO cells planted at a single cell theoretical count.

#### **What is the overall protein content of EX-CELL CD CHO?**

EX-CELL CD CHO contains approximately 0.1 mg/L of recombinant protein.

#### **What are the molecular weights of the proteins in this medium?**

All proteins are < 10 kDa.

#### **What is EX-CELL CD CHO's buffering system?**

EX-CELL CD CHO is buffered using a bicarbonate and MOPS system.

#### **What are the best methods for protein purification for product produced from EX-CELL CD CHO?**

EX-CELL CD CHO is serum-free, animal-component free and chemically defined. It contains a low level of recombinant protein (0.1 mg/L), therefore downstream processing including protein purification becomes much more amenable. Protein purification can involve a number of steps to achieve a satisfactory yield of final product. These methods can include ultra-filtration and/or chromatography including affinity, size and charge separation. Most likely a combination of these will need to be utilized to attain acceptable yields.

#### **Can EX-CELL CD CHO be used for cryopreservation?**

##### **What are recovery rates?**

Yes, CHO cells can be frozen in EX-CELL CD CHO without the reintroduction of serum or other protein components. Healthy, viable (> 90%) cultures in logarithmic growth should be used.

A freezing medium consisting of 45% fresh medium, 45% conditioned medium and 10% dimethyl sulfoxide (DMSO) can be used. Alternatively, cells can be frozen in 90% fresh medium and 10% DMSO. Please refer to the SAFC Product Information Sheet for each EX-CELL CD CHO product for more detailed information.

Recovery rates typically exceed 90%.

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