IS CHO-CD XP Culture System

Catalog #	Product	Size
91120 *	IS CHO-CD XP	1 L liquid
91121 *	IS CHO-CD XP with IS Hydrolysate Blend XP	1 L liquid
91122 *	IS CHO Feed-CD XP	500mL liquid
94109 *	IS Hydrolysate Blend XP	10g powder
94110	IS CHO-CD XP – Powder	10 L powder
94113 *	IS CHO-CD XP Powder with IS Hydrolysate Blend	10 L powder
94114 *	IS CHO Feed-CD XP Powder	5 L powder

* Available only as a made-to-order product.

NOTE: Custom packaging and sizes available upon request.

Intended Use

For further manufacturing use. The IS CHO Culture System product family is comprised of a set of media formulations of a basal medium, a feed medium, and a hydrolysate supplement for producing recombinant proteins in high-density CHO cell cultures using a variety of manufacturing methods including batch and fed-batch culture processes.

Product Description

The IS CHO Culture System is comprised of individual products including IS CHO-CD XP basal and IS CHO Feed-CD XP feed media to support culture of recombinant Chinese Hamster Ovary (CHO) cells. This system is designed to support batch, fed-batch, and perfusion culture processes. The IS CHO Culture System components can be combined as needed and are provided for convenience and flexibility. All IS CHO Culture System components are fully compatible with other Irvine Scientific CHO culture media.

IS Hydrolysate Blend XP is available as a performance enhancing supplement that can be provided as a separate powder supplement or included in IS CHO-CD XP with Hydrolysate Blend XP powder or liquid basal media.

The IS CHO Culture System Kit is an evaluation kit comprised of 1L IS CHO-CD XP (91120), 1L IS CHO-CD XP with Hydrolysate Blend XP (91121), 500mL IS CHO Feed-CD XP (91122), and 10g IS Hydrolysate Blend XP (94109).

Formula

All IS CHO Culture System components are serum-free and animal component-free. All components are provided without L-Glutamine. IS CHO-CD XP and IS CHO-CD XP with Hydrolysate Blend XP liquid media are provided with 2.2g/L sodium bicarbonate. IS CHO-CD XP and IS CHO Feed-CD XP are chemically-defined. IS CHO FEED-CD medium is a concentrated feed supplement for fed-batch culture. IS Hydrolysate Blend XP is made up of ultra-filtered hydrolysates from multiple plant sources and can be used as a supplement to basal and/or feed media. All components contain no antibiotics or antimycotics.

Quality Assurance

Quality Control testing is performed on every lot of each IS CHO Culture System component. Results are reported on lot-specific Certificates of Analysis that are available upon request. All liquid IS CHO Culture System components are sterile-filtered using a 0.1µM filter and are compliant with current USP <71> and 21CFR, part 610.12 regulations.

Storage Instructions and Stability

Handle liquid IS CHO Culture System components using aseptic technique to avoid contamination. Minimize exposure to light for all liquid components. Store IS CHO-CD XP, IS CHO-CD XP with Hydrolysate Blend XP, and IS CHO Feed-CD XP at 2-8°C. Store IS Hydrolysate Blend XP at 15-30°C. Do not use IS CHO Culture System components after the expiration date on the label.

Recommended Materials Not Provided

Liquid IS CHO Culture System media are provided without L-Glutamine. Powder IS CHO Culture System media are provided without L-Glutamine and Sodium Bicarbonate. These materials are required for effective culture of most CHO cell lines (please see the Directions For Use section of this document). L-Glutamine Solution (200mM, Catalog# 9317) is recommended for use with Irvine Scientific media. For preparation of powder culture media, high quality water such as Water for Injection (WFI, Catalog# 9309) is recommended.

Precautions

Do not use any bottle of liquid medium which shows evidence of particulate matter or cloudiness. Minimize exposure to light for all liquid components.

Directions for Use

General Directions

IS CHO-CD XP and IS CHO-CD XP with Hydrolysate Blend XP are intended for use as basal media in batch, fed-batch, and perfusion culture systems. They are designed to be used for culture processes with controlled pH or environmental CO_2 set at 5-10% CO_2. The media are supplied without L-Glutamine and may be supplemented with L-Glutamine as needed. The recommended procedure is to aseptically add 40mL/L L-Glutamine Solution (200mM; Catalog# 9317) for a final concentration of 8mM L-Glutamine in the basal medium. Note that for GS selection systems L-Glutamine is typically not added. Liquid media are provided with sodium bicarbonate and powders without. It is recommended that 2.2g/L sodium bicarbonate be added to powder media upon dissolution for proper pH control.

IS CHO FEED-CD XP is intended for use in fed-batch cultures as a feed supplement for either IS CHO-CD XP or IS CHO-CD XP with Hydrolysate Blend XP basal media. IS CHO Feed-CD XP is provided at pH ~7.0 for ease of use. A basic fed batch strategy is provided below. Process development to determine optimal feeding volumes and timing should be conducted to maximize cell growth and production for a specific cell line and process. Other materials may be added to the feed supplement including L-Glutamine, Hydrolysates and additional Glucose for increased performance.

Basic Feed Strategy using IS CHO Feed-CD XP:

1. Determine total working volume (wv) of culture.
2. Inoculate the cells in basal medium at ≤ 90% of the total wv.
3. On culture days 2 through 6, feed 2% of the total wv with IS CHO FEED-CD XP (total 10% wv). The feed can be added in a single bolus for each daily feeding event.

2L Working Volume Bench Scale Bioreactor Example:

1. Total working volume equals 2L.
2. Inoculate cells in 1.8L IS CHO-CD XP at appropriate density.
3. On culture days 2 through 6, feed 40 mL/day with IS CHO FEED-CD XP (total 200mL, 10% wv).

IS Hydrolysate Blend XP is intended for use as a supplement for batch, fed batch, or perfusion cultures. It can be used to supplement both basal and feed media. It is recommended that 6g/L IS Hydrolysate Blend XP be added to basal media. Process development can be conducted to determine a more optimal concentration for a specific cell line and process.

Hydration of Powder Media

IS CHO-CD XP (94110) or IS CHO-CD XP with Hydrolysate Blend XP (94113)

1. Add 980mL/L WFI or cell culture grade water to appropriate size mixing vessel.
2. Add appropriate amount of media powder:

   For IS CHO-CD XP add 21.74g/L IS CHO-CD XP powder.

   For IS CHO-CD XP with Hydrolysate Blend XP add 27.74g/L IS CHO-CD XP with Hydrolysate Blend XP powder.
3. Mix moderately until dissolved (approximately 30 minutes).
4. Optional: Add IS Hydrolysate Blend XP if desired and mix moderately until dissolved (approximately 15 minutes).
5. Add 2.2g/L Sodium Bicarbonate.
6. Mix moderately until dissolved (approximately 10 minutes).

   Note: Minimize mixing to avoid CO_2 off-gassing that can result in pH changes.
7. Check pH and osmolality.

   For IS CHO-CD XP pH range should be pH 6.9-7.3 and osmolality should be 280-320mOsm/Kg.

   For IS CHO-CD XP with Hydrolysate Blend XP pH range should be pH 7.0-7.4 and osmolality should be 290-350mOsm/Kg.
8. Filter sterilize the media using a 0.1µM filter system into appropriate containers.
9. Optional: Aseptically add 40mL/L (or appropriate amount) of L-Glutamine Solution (200mM; Catalog# 9317).
10. Store liquid IS CHO-CD XP media at 2-8°C and minimize exposure to light.

IS CHO Feed-CD XP (94114)

1. Add 950mL/L WFI or cell culture grade water to appropriate size mixing vessel.
2. Add 74.16g/L IS CHO Feed-CD XP powder.
3. Mix moderately for 30 minutes.
4. Optional: Add additional glucose or other supplement as needed and mix for 30 additional minutes.
5. Optional: Add IS Hydrolysate Blend XP if desired and mix moderately for 30 additional minutes.
6. Add 1.6mL/L 5N NaOH.
7. Mix moderately for 4 hours (or until all powder is completely dissolved).
8. Check pH and osmolality. pH range should be pH 6.8-7.5 and osmolality should be 490-560mOsm/Kg.
9. Filter sterilize the feed media using a 0.1µM filter system into appropriate containers.
10. Store liquid IS CHO-CD XP media at 2-8°C and minimize exposure to light.
11. Note: pH should stabilize at approximately pH 6.8-7.2 after 1 week of storage.

Adaptation

The two strategies outlined below are typically performed concurrently. If direct adaptation is successful, then the sequential method can be stopped. The methods outlined below can be used to adapt from a serum-free media into IS CHO Culture System media or to adapt adherent cell lines in serum containing media to suspension culture into IS CHO Culture System media. Note that adaptation with some cell lines can be difficult and can often require considerable patience and time.

A. Direct Adaptation from Serum-Supplemented Media to IS CHO-CD XP or IS CHO-CD XP with Hydrolysate Blend XP

Note: In many cases, CHO cells may be sub-cultured from a serum-supplemented medium (e.g., Ham's F-12/DME + 10% FBS) directly into IS CHO-CD XP or IS CHO-CD XP with Hydrolysate Blend XP.

1. Dispense IS CHO-CD XP or IS CHO-CD XP with Hydrolysate Blend XP medium into a culture vessel and equilibrate to 37°C and 5% CO_2.
2. Passage CHO cells from serum-supplemented culture into the medium at $3x10^5$ viable cells/mL. It is important that cells be in the logarithmic phase of growth with at least 90% viability before passaging.
3. Incubate cultures at 37°C and 5% CO_2 until the viable cell density reaches $>1x10^6$ cells/mL.
4. Subculture into fresh medium at a density of $2x10^5$ viable cells/mL.
5. Maintain cells in appropriate medium for several passages, subculturing twice weekly to allow complete adaptation and assure optimum performance.

B. Sequential adaptation from serum-supplemented media to IS CHO-CD XP or IS CHO-CD XP with Hydrolysate Blend XP

Note: Sequential adaptation may be used if direct adaptation is troublesome.

1. Dispense the original serum-supplemented medium and IS CHO-CD XP or IS CHO-CD XP with Hydrolysate Blend XP medium in a 3:1 ratio into an appropriate culture vessel and equilibrate to 37°C and 5% CO_2.
2. Passage CHO cells from serum-supplemented culture into a 3:1 ratio of serum-supplemented medium to IS CHO-CD XP medium at $3x10^5$ viable cells/mL. It is important that cells be in the logarithmic phase of growth with at least 90% viability before passaging.
3. Incubate cultures at 37°C and 5% CO_2 until the viable cell density reaches $1x10^6$ cells/mL.
4. Subculture at $3x10^5$ cells/mL starting density into fresh medium prepared in a 2:1 ratio of original serum-supplemented medium to IS CHO-CD XP medium.
5. Repeat steps 3 and 4 with sequential dilution ratios of 1:1, 1:2, 1:4, and 0:1 of the original serum-supplemented medium and IS CHO-CD XP. If the cells look unhealthy or the growth rate declines significantly at a particular step of adaptation, maintain the cells for an additional passage in the media ratio of the previous step before sub-culturing into the next ratio.
6. Maintain cells in IS CHO-CD XP for several passages, sub-culturing twice weekly to allow complete adaptation and assure optimum performance.

Cryopreservation

Viable cell banks may conveniently be created by freezing cells in 90% IS CHO-CD XP or IS CHO-CD XP with Hydrolysate Blend XP + 10% DMSO. Most cell lines can be successfully banked using fresh media and DMSO. No other additions are necessary.

A. Freezing

1. Use cultures that are in logarithmic growth with high viabilities (>90%).
2. Centrifuge cells for 5 minutes at 200g.
3. Re-suspend the cells in cold (2-8°C) 90% fresh media and 10% DMSO to a density of $1\text{-}2x10^7$ viable cells/mL. A mix of 45% fresh media, 45% conditioned media, and 10% DMSO may be used for more sensitive cell lines.
4. Aliquot into sterile cryovials.
5. Gradually lower the temperature of the vials to below -80°C at a rate of -1°C/minute.
6. Store vials in a liquid nitrogen cryopreservation vessel.

B. Thawing

1. Thaw frozen vial rapidly in a 37°C water bath.
2. Transfer the cell suspension to a culture flask with fresh IS CHO-CD XP or IS CHO-CD XP with Hydrolysate Blend XP medium to achieve an initial cell density of $3x10^5$ viable cells/mL.
3. Incubate cultures at 37°C and 5% CO_2 until the viable cell density reaches $1x10^6$ cells/mL.
4. Sub-culture into fresh medium at $2x10^5$ cells/mL starting density.

Related Products

Catalog #	Product	Size
9197 *	IS CHO-V	1 L liquid
9198 *	IS CHO-V-GS	1 L liquid
91119 *	IS CHO-CD	1 L, 10 L, 50 L liquid
91100 *	IS CHO-CD4	1 L liquid
91108 *	IS CHO Feed-CD	500mL, 100 L, 200 L liquid
9317	L-Glutamine Solution (200mM)	100mL, 500mL liquid
96850 *	IS Hydrolysate Blend	1 kg liquid; 6gm, 60gm, powder
96857 *	Soy Hydrolysate UF	10gm, 100gm, 500gm, 1 kg powder
96863 *	Yeast Hydrolysate UF	10gm, 1 kg, 15kg powder

* Available only as a made-to-order product

All of these products are compatible with the IS CHO Culture System. All listed media are serum-free and free of animal-origin components. IS CHO-CD, IS CHO-CD4, IS CHO Feed-CD, and L-Glutamine Solution (200mM) are chemically defined.

Technical Support

CONTACT US

For more information or assistance contact Customer Service at:

- Email: fisitmrequest@fujifilm.com
- Direct line: +1 800 577 6097

WEBSITE RESOURCES

Visit the website at www.irvinesci.com for technical resources and information including:

- Safety Data Sheets (SDS)
- Certificate of Analysis (CoA) (when available)
- FAQs
- Product literature
- Complete list of offices and contact information by country

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