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Xtreme™ CHO Feed Chemically Defined and Protein-free Liquid and Powdered Formats

Instructions for use

Contents:

I. Introduction

Xtreme™ CHO Feed CD is a serum-free, chemically defined medium for the feeding of CHO cells in serum-free conditions. It is designed to work with PowerCHO™ 2 but works very well with Lonza PowerCHO™ 1 and ProCHO™ 4 and 5, and many other serum-free CHO media on the market. Cells can therefore be directly transitioned from any medium–feed system to Xtreme™ CHO Feed with little to no adaptation time.

NOTE: Application-specific procedures may be used in place of this general protocol.

II. Materials

- CHO cell line
- Lonza CHO Medium, for example PowerCHO™ 2 (BP12-771Q or BELN12-771Q)
- Lonza Xtreme™ Feed Medium P/N BE02-056Q (liquid) or powdered equivalents P/N VPW-098D or VPW-098Q
- L-glutamine, 200mM – Lonza P/N: BEBP17-605E
- 125 mL and 250 mL polycarbonate vented Erlenmeyer flasks

III. Major equipment / capabilities

- Incubator – 37˚C, 5% CO_2, 95% relative humidity
- Shaker platform (in the incubator) – set to 100-110 rpm (or speed necessary for CHO cell line of interest)
- Metabolite analyzer for at least glucose, (lactate, glutamine, and glutamate secondary requirements)
- ViCell, Cedex, or other automated cell counter. Alternatively a hemacytometer can be used.
- Protein titer assay

IV. Reconstitution of Xtreme™ Feed Powder

Reconstitute the powder as follows to make 10 L or 200 L of liquid Xtreme™ CHO Feed.

1. Select a suitable container to accommodate the final volume of medium. Fill to 90% final volume with distilled water at 20° C, i.e. 9 L or 180 L.
2. While stirring, add 1 container of the basal powder VPW-098D (10 L) or VPW-098Q (200 L)
3. Rinse the powder container with a small volume of water and add to the vessel.
4. Continue mixing until the powder is completely dissolved.
   Once the basal powder is completely dissolved, add to the vessel ferric citrate solution and $NaHCO_3$ following the table below:

Supplement table

	Fe citrate	$NaHCO_3$
Part #	02-059	15-613I
10 L	98.76 mL	21.5 g
200 L	1975 mL	430 g

5. Stop mixing as soon as dissolution is complete. Adjust pH to approximately 6.9 with NaOH (not supplied). Add water to reach the final volume.
6. Optional: Check the osmolality of the solution. Without adjustment it should be in the range of 450 mOsm/kg.
7. Immediately filter into a sterile container using a 0.2 µM absolute filter. We recommend using a Sartopure™ 0.65µM pre-filter or equivalent connected to a Sartopore™ 0.2µM filter or equivalent. Size of pre-filter must be adapted according to batch size.
8. Store reconstituted medium at 2 - 8°C in the dark.

V. Storage conditions for individual components

- Xtreme™ Feed Basal Powder should be stored at 2 - 8°C in dry conditions.
- Fe citrate (02-059) should be stored at 15 - 30°C.

VI. Fed-batch strategy development

Objective: to test Lonza CHO Medium and Xtreme™ CHO Feed in a fed-batch process development study.

Adaptation into serum-free media and scale-up

The CHO cell culture should be adapted to a suspension, serum-free culture medium. If the culture is currently in a serum-containing medium, the cells should be weaned into a serum-free medium for at least three passages prior to initiating the study. A serum-free CHO medium to use for weaning is Lonza PowerCHO™ 2 Medium P/N BP12-771Q or BELN12-771Q

Media preparation

1. Receive one 1 L bottle of Lonza PowerCHO™ 2 Medium as well as one 1 L bottle of Lonza Xtreme™ Feed. Store at 2 - 8°C protected from light.
2. Completely thaw L-glutamine, 200 mM, Lonza P/N BEBP17-605E.

   (If necessary,) aseptically supplement the Lonza PowerCHO™ 2 Medium with 4-8 mM L-glutamine

Adaptation into PowerCHO™ 2 Medium

NOTE: Depending on the growth rate and adaptation of the particular cell line, cells may be thawed directly into Lonza PowerCHO™ 2 Medium, followed by three passages post-thaw prior to assay inoculation.

1. Count and record the culture viable cell densities on the first adaptation day.
2. Calculate the volume of cells needed to inoculate the adaptation condition. 1.0 x10^7 cells will be needed for the fed-batch process development study. This is 400,000 cells/mL x 25mL.

$$\text{Volume of cells} = \frac{\text{Total cells needed (cells)}}{\text{Density of scale - up flask (cells/mL)}}$$

3. Calculate the volume of fresh (pre-warmed) medium to add to each new vessel.

Medium needed (mL) = Target total volume – Volume of cells

4. Aseptically pipette medium and cells into a new, labeled Erlenmeyer flask.
5. Perform a Day 0 cell count to confirm the correct seeding density has been achieved.
6. Place Erlenmeyer flask on a shaker platform set at 100-110 rpm in a humidified 37˚C, 5% CO_2 incubator.
7. Allow flask to incubate for 3 days.

NOTE: Culture duration and incubator settings may vary depending on the growth rate of the particular cell line. Timing of passages should be adjusted to existing protocols.

Passaging cells

<u>Direct media adaptation:</u>

1. Count and record the viable cell densities on the passage day.

2. Calculate the volume of cell suspension needed to seed the next passage.

$$\text{Volume of cells} = \frac{\text{Target volume (mL) x Target Density (cells/mL)}}{\text{Cell Density (Viable cells/mL)}}$$

3. Calculate the volume of fresh (pre-warmed) medium to add to each new vessel.

Medium needed (mL) = Target total volume – Volume of cells

4. Pipette medium and cells into new, labeled Erlenmeyer flask.
5. Place flasks in the incubator at 37˚C, 5% CO_2 for 3-4 days.
6. Culture cells in Lonza PowerCHO™ 2 Medium for a minimum of 2 (preferably at least 3) passages to ensure cells have adapted to the medium.
7. Most cell lines may be directly adapted into Lonza PowerCHO™ 2 Medium. Cells should be seeded between 1 and 5 $\times 10^5$ cells/mL such that they can be sub-cultured when densities reach between 2 and 4 $\times 10^6$ cells/mL in 2-4 days with greater than 90 % viability. Adaptation is complete when an acceptable doubling time is achieved and viability is greater than 90% over at least 2 passages.
8. Generate a master cell bank for cells adapted to Lonza PowerCHO™ 2 Medium.

NOTE: To seed the fed-batch process development studies in Table 1, at least 120 mL of cells will be needed at 2,000,000 cells/mL

VII. Fed-batch study initiation for shake flasks

1. Day 0 (Preferably Friday)

a. Count cells in passage and calculate volume of cells to seed 60 mL of medium at 300,000 cells/mL into 250 mL Erlenmeyer flasks.

b. Pipette cells from passage flasks into the respective assay seeding flasks for each condition listed in Table 1.

c. Once seeded, count cells to confirm that the correct seeding density has been achieved.

d. Sample each culture and assay for Glucose, Lactate, L-glutamine and L-glutamate.

2. Days 3 – 14

a. Sample flasks at minimum on days 3, 5, 7, 10, 12, and 14 (preferably daily) for viable cell density and protein titer.

b. Count and record the viable cell density for each culture until cell viability drops below 50% at which time the culture can be sampled for protein titer and discarded.

c. Feed flasks with Lonza Xtreme™ Feed according to one or more of example feeding strategies in Table 1. Total feed volume added is typically between 30 - 50% of initial culture volume for most systems (though some cell lines have required as little as 20% feed volume).

d. Sample each culture for Glucose, Lactate, L-glutamine, L-glutamate and any other metabolites that can be measured.

e. Supplement cultures with additional glucose to prevent glucose depletion. For example, if cultures are sampled every other day, when glucose concentrations are below 4 g/L, supplement up to 6 g/L. Also account for glucose addition by the feed which contains 30 g/L glucose and for example would add 3 g/L with a 10% feed. Some cell lines may consume as much at 2 - 3 g/L glucose per day at peak density.

Table 1: Example fed-batch process development screening studies

Condition number	Feed percent of initial volume per day													
	3	4	5	6	7	8	9	10	11	12	13	Total	Basal	Feed
1	10		10		10		10					40	PowerCHO™ 2 Medium	Xtreme™ Feed
2	5	5	5	5	5	5	5	5				40		
3		10		10		10		10				40		
4		5	5	5	5	5	5	5	5			40		
5	10		10		10		10		10			50		
6	5	5	5	5	5	5	5	5	5	5		50		
7		10		10		10		10		10		50		
8		5	5	5	5	5	5	5	5	5	5	50		
9													PowerCHO™ 2 Medium	Glucose
10													PowerCHO™ 2 Medium	Control
11													Customer control	

Condition number	Feed percent of initial volume per day													
	3	4	5	6	7	8	9	10	11	12	13	Total	Basal	Feed
1	8		8		8		8		8			40	PowerCHO™ 2 Medium	Xtreme™ Feed
2	4	4	4	4	4	4	4	4	4	4		40		
3		8		8		8		8		8		40		
4		4	4	4	4	4	4	4	4	4	4	40		
5	10		10		10		10		10			50		
6	5	5	5	5	5	5	5	5	5	5		50		
7		10		10		10		10		10		50		
8		5	5	5	5	5	5	5	5	5	5	50		
9													PowerCHO™ 2 Medium	Glucose
10													PowerCHO™ 2 Medium	Control
11													Customer control	

Condition number	Feed percent of initial volume per day													
	3	4	5	6	7	8	9	10	11	12	13	Total	Basal	Feed
1	10		10		10		10					40	PowerCHO™ 2 Medium	Xtreme™ Feed
2	5	5	5	5	5	5	5	5				40		
3		10		10		10		10				40		
4		5	5	5	5	5	5	5	5			40		
5	8		8		8		8		8			40		
6	4	4	4	4	4	4	4	4	4	4		40		
7		8		8		8		8		8		50		
8		4	4	4	4	4	4	4	4	4	4	50		
9	10		10		10		10		10			50		
10	5	5	5	5	5	5	5	5	5	5		50		
11		10		10		10		10		10		50		
12		5	5	5	5	5	5	5	5	5	5	50		
13													PowerCHO™ 2 Medium	Glucose
14													PowerCHO™ 2 Medium	Control
15													Customer control	

NOTE: Total volumes and schedules can be adjusted based on consumption and historical feeding strategies.

VIII. Ordering information

Cat. no.	Product	Format	Size
BE02-056Q	CHO Xtreme™ Feed Chemically Defined	Liquid	1 L
VPW-098D	XtremeFeed™– Chemically Defined and Protein-free Basal Powder	Powder	10 L
VPW-098Q	XtremeFeed™– Chemically Defined and Protein-free Basal Powder	Powder	200 L
BE02-059E	Ferric Citrate	Liquid	100 mL
BE02-059F	Ferric Citrate	Liquid	500 mL
BE02-059Q	Ferric Citrate	Liquid	1 L

***Products are for Research Use Only**

This product is not intended for direct therapeutic use in humans.

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IX. Related products

Cat. no.	Product	Size
BEBP17-605E	L-Glutamine 200 mM solution	100 mL
15-613I	Sodium Bicarbonate Powder	500 g
17-613E* BE17-613E (EU-only),*	Sodium Bicarbonate 7.5% Solution	100 mL
BEBP17-855E	ProHT Supplement (100x)	100 mL
BP12-770Q	PowerCHO™ 1 Serum-free Medium	1 L
BP12-771Q BELN12-771Q	PowerCHO™ 2 Serum-free Medium	1 L
BE12-771P10	PowerCHO™ 2 Serum-free Medium	10 L
BE12-771P20	PowerCHO™ 2 Serum-free Medium	20 L
BE15-771ND	PowerCHO™ 2 Serum-free Medium Powder	10L
BE15-771NF	PowerCHO™ 2 Serum-free Medium Powder	50L
BE15-771NJ	PowerCHO™ 2 Serum-free Medium Powder	100L
BEBP12-769E	TheraPEAK™ ProFreeze™ Freezing Medium, CD, NAO 2x	100mL

X. Product Use Statement

GMP PRODUCTS ARE INTENDED FOR RESEARCH or FURTHER MANUFACTURING USE ONLY.

This product is not intended for direct therapeutic use in humans.