Lonza Walkersville, Inc.
Walkersville, MD 21793-0127 USA
U.S. Scientific Support: 800 521 0390
scientific.support@lonza.com
EU/ROW Scientific Support: +32 87 321 611
scientific.support.eu@lonza.com
Document # PA-0262 / CD-MN046a
bioscience.lonza.com

Lonza eCHO™ Basal and Feed Media

Instructions for use in shake flasks

Introduction

The Lonza eCHO™ Basal Medium and feed medium are non-animal origin, protein-free, and chemically defined products. Instructions for adaptation into Lonza eCHO™ Basal Medium, passaging cells in this medium, and setting up a fed-batch feed strategy development assay are included in this document.

NOTE: Procedures specific and/or optimized to a particular cell line should override and be used in place of general cell culture suggestions contained in this document.

Safety statements

GMP PROUCTS ARE INTENDED FOR RESEARCH or FURTHER MANUFACTURING USE ONLY.
This product is not intended for direct therapeutic use in humans.

Materials

- CHO cell line
- Lonza eCHO™ Basal Medium P/N BEBP12-933Q
- Lonza eCHO™ Feed Medium P/N BEBP12-932Q
- L-glutamine, 200mM – Lonza P/N: BEBP17-605E
- 125 mL and 250 mL polycarbonate vented Erlenmeyer flasks

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

www.lonza.com

U.S. Scientific Support: 800-521-0390
scientific.support@lonza.com

EU Scientific Support: 32 (0) 87 321 611
scientific.support.eu@lonza.com

Fed-batch feed strategy development

Objective: To test Lonza eCHO™ Basal and Feed Media 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 eCHO™ Basal Medium P/N BEBP12-933Q.

Media preparation

1. Receive one 1 L bottle of Lonza eCHO™ Basal Medium as well as one 1 L bottle of Lonza eCHO™ Feed Medium. Store at 2°C to 8°C protected from light.
2. Completely thaw and dissolve L-glutamine, 200 mM, Lonza P/N BEBP17-605E.
3. (If necessary,) aseptically supplement the Lonza eCHO™ Basal Media with 4-8 mM L-glutamine.

Adaptation into Lonza eCHO™ Medium

NOTE: Depending on the growth rate and adaptation of the particular cell line, cells may be thawed directly into Lonza eCHO™ 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 400,000cells/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.

Passage cells

Direct media adaptation:

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.

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

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 eCHO™ 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 eCHO™ Basal 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 eCHO™ 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

Product use statement

GMP PROUCTS ARE INTENDED FOR RESEARCH or FURTHER MANUFACTURING USE ONLY.
This product is not intended for direct therapeutic use in humans.

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 eCHO™ Feed Medium 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

Condition	Feed Percent of Initial Volume per Day													
Number	3	4	5	6	7	8	9	10	11	12	13	Total	Basal	Feed
1	10		10		10		10					40	eCHO™ Basal Medium	eCHO™ Feed Medium
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													eCHO™	Glucose
10													eCHO™	Control
11													Customer Control	

Condition	Feed Percent of Initial Volume per Day													
Number	3	4	5	6	7	8	9	10	11	12	13	Total	Basal	Feed
1	8		8		8		8		8			40	eCHO™ Basal Medium	eCHO™ Feed Medium
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													eCHO™	Glucose
10													eCHO™	Control
11													Customer Control	

Condition	Feed Percent of Initial Volume per Day													
Number	3	4	5	6	7	8	9	10	11	12	13	Total	Basal	Feed
1	10		10		10		10					40	eCHO™ Basal Medium	eCHO™ Feed Medium
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		40		
8		4	4	4	4	4	4	4	4	4	4	40		
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													eCHO™	Glucose
14													eCHO™	Control
15													Customer Control	

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

Related products

L-Glutamine 200 mM solution
BEBP17-605E 100 mL

ProHT supplement 100x
BEBP17-855E 100 mL

ProFreeze™ NAO 2x

12-769E 100 mL