Lonza eCHO™ Basal and Feed Powder Media
Instructions for use

Chemically defined, serum-free eCHO™ Basal and Feed Powder Media
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Introduction
The Lonza eCHO™ Basal Medium and Feed Medium are non-animal origin, protein-free, and chemically defined products. Instructions for reconstitution of powder, 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.

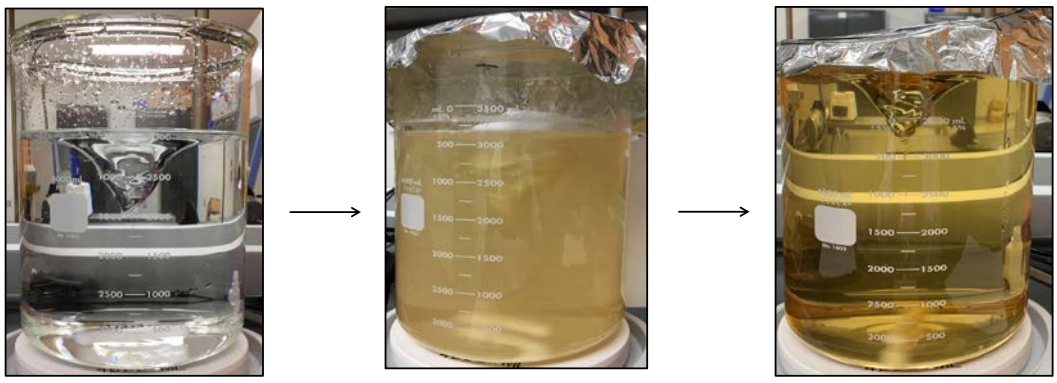
NOTE: Procedures specific and/or optimized to a particular cell line should override and be used in place of general cell culture suggestions contained in this document.

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

Reconstitution of eCHO™ Basal Medium

1. Choose the vessel and agitation method that is appropriate for the batch size to be prepared.
2. Confirm that the quality of the water to be used is sufficient for preparing the media and that the vessel and stir bar (or agitator) are clean and rinsed with the WFI quality water to be used in the media preparation.
3. Fill the vessel to 90% of the final volume with WFI water.
4. Start mixing the water at a speed to generate a vortex that is 30 – 50% of the height of the vessel (see image, below).

Preparation of 4 L eCHO™ Basal Liquid Media from powder media



Before the addition of powder media

After the addition of powder media

pH adjusted and make up the volume to 4 L (Before sterile filtration)

5. Slowly add the eCHO™ Basal Powder Medium to the vessel at 30.14 g/L.
6. It should require 15 to 20 minutes for the powder medium to fully dissolve.

NOTE: If necessary, rinse the powder off the walls of the vessel with a small volume of the same WFI water.

7. After all the powder clumps have been dissolved into the solution, mix for an additional 30 minutes (minimum) to ensure that the mixture is homogeneous.

8. Slowly adjust the pH to 7.0 with 5 N Sodium hydroxide. This should typically require between 1 and 1.3 mL NaOH/L medium.

9. Continue mixing for an additional 10 minutes to ensure pH homogeneity and stability.

10. Dilute to the final volume with room temperature WFI water.

11. Mix for a minimum of 10 minutes to ensure homogeneity.

12. Sterilize by filtration using a 0.2 micron filter. Note: Sartorius sterile filter Sartopore™ Capsule 0.2µM cat# 5441307H5--OO--B may be used.

13. The final expected pH range is 6.8 - 7.2, and the final osmolality is about 330 (no final osmolality adjustment is required).

Reconstitution of eCHO™ Feed Medium

1. Choose the vessel and agitation method that is appropriate for the batch size to be prepared.
2. Confirm that the quality of the water to be used is sufficient for preparing the media and that the vessel and stir bar (or agitator) are clean and rinsed with the WFI quality water to be used in the media preparation.
3. Fill the vessel to 80% of the final volume with WFI water.
4. Start mixing the water at a speed to generate a vortex that is 50 – 70% of the height of the vessel (see image, below).

Preparation of 4 L eCHO™ Feed Liquid Media from powder media

Before the addition of powder media | **After the addition of powder media** | **pH adjusted and make up the volume to 4 L (Before sterile filtration)**

Images from start to finish

5. Add 5 N Sodium Hydroxide at 15 mL/L final volume.

6. Slowly add eCHO™ Feed Medium to the vessel at 72.8 g/L.
7. Mix until all the powder is dissolved. It should require around 45 minutes for the powder to dissolve at the appropriate mixing speed and the solution will appear cloudy.

NOTE: If necessary, rinse the powder off the walls of the vessel with a small volume of the same WFI water.

8. Add an additional 15 mL/L final volume 5 N Sodium Hydroxide. NOTE: pH will be approximately 8.2 - 8.4 before addition and 9.1 - 9.3 afterward.
9. Mix for an additional 30 minutes to ensure a homogeneous pH.
10. Slowly adjust the pH to 7.0 with 5 N Hydrochloric Acid. This will typically require 15 - 20 mL/L of 5 N HCl.
11. Continue mixing for a minimum of 10 minutes longer.
12. Dilute to final volume with WFI water and mix for a minimum of 10 minutes longer.
13. Sterilize by filtration using a 0.2 micron filter. Note: Sartorius sterile filter Sartopore™ Capsule 0.2 µM (Cat# 5441307H5--OO—B) may be used.
14. The final expected pH range is 6.8 - 7.2, and the final expected osmolality range is 750 – 850 (no final osmolality adjustment is required).

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 eCHO™ 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.

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

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 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

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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.

Products

eCHO™ Basal Powder

BE15-933Q	10L
BE15-933D	50L

eCHO™ Feed Powder

BE15-932Q	10L
BE15-932F	50L

eCHO™ Basal Liquid

BEBP12-933Q	1L

eCHO™ Feed Liquid

BEBP12-932Q	1L

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

Table 1: Example fed-batch process development screening studies

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.