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PowerFeed™ A - Liquid and Powder Formats

Instructions for powder reconstitution and culturing in shake flasks

I. Introduction

The Lonza PowerFeed™ A is non-animal origin, protein-free, and chemically defined media feed for CHO cells. Instructions for powder reconstitution, cell adaptation, passaging, and fed-batch strategy are included in this document.

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

II. Powder kit components

BE15-044D, F, J, Q and L are 10, 50, 100, 200 and 500L PowerFeed A powder kit, made of:

	Part code	(BE)15-044				
		D	F	J	Q	L
Basal Powder	VPW-095D (10L-527.95g)	1	-	-	-	-
	VPW-095F (50L-2639.76 g)	-	1	-	-	-
	VPW-095J (100L-5279.51 g)	-	-	1	-	-
	VPW-095Q (200L-10559.03 g)	-	-	-	1	-
	VPW-095L (500L- 26397.57 g)	-	-	-	-	1
Ferric Citrate Solution	BE02-059E (100 mL)	2	-	-	-	-
	BE02-059Q (1L)	-	1	2	4	10

III. Instructions for powder reconstitution

Powder has to be reconstituted as follows to obtain 10, 50, 200 or 500 L of liquid PowerFeed™ A:

1. Select a suitable vessel as close in size to the final volume as possible. Fill it with 90% of the final volume with deionized or distilled water at 20°C (i.e. 9. 45, 90, 180 or 450 L).
2. While gently stirring, add 1 container of VPW-095. Wait 10 minutes to dissolve the chemicals in acidic conditions. Adjust the pH between 6.5 to 6.9 to aid in the dissolution of the powder. Mix until dissolution is complete (30 minutes maximum.)
3. Rinse the powder container with a small volume of water and add to the solution.
4. Add the ferric citrate solution to the vessel using the following instructions:

	10 L	50 L	100 L	200 L	500 L
Ferric Citrate	186.9 mL	934.6 mL	1869.2 mL	3738.3 mL	9346 mL

5. Stop mixing as soon as dissolution is completed. Re-adjust pH with NaOH or HCl if needed between 6.5 and 6.9. Add water to reach the final volume.
6. Check osmolality. It should be between 550 and 580 mOsm/kg. If too low, NaCl can be added to the medium following this formula:

(Target osmo-current osmo) x 30 = mg NaCl per liter of medium to add

7. Immediately filtrate on 0.2 µM absolute filter into a sterile container. We recommend using Sartopure 0.65 µM prefilter or quivalent connected to a 0.2 µM filter or equivalent. Size of prefilter must be adapted to batch size. Store liquid medium at 2 - 8°C in the dark.

NOTE: Lonza does not recommend using only part of the powder containers. However, if this is absolutely required, VPW-095 powders should be used at 52.795 g/L with 18.69 mL of BE02-059.

IV. Storage

VPW-095 powders should be stored at 2 - 8°C in dry conditions.

BE02-059 should be stored at 15 - 30°C and is highly sensitive to light (properties change after just a few hours of light exposure).

V. Materials

- CHO cell line
- Lonza CHO Medium for example PowerCHO™ 2
- Lonza PowerFeed™ Powder
- L-glutamine, 200mM – Lonza P/N: BEBP17-605E
- 125 mL and 250 mL polycarbonate vented Erlenmeyer flasks

VI. 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

VII. Fed-batch strategy development

Objective: To test Lonza CHO Medium and PowerFeed™ Medium 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.

VIII. 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 400,000cells/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.

IX. Passaging 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 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 ×10^5 cells/mL such that they can be sub-cultured when densities reach between 2 and 4 ×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 CHO 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

X. 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 PowerFeed™ A 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.

XI. 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	PowerFeed™ A
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	PowerFeed™ A
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	PowerFeed™ A
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.

XII. Ordering information

www.bioscience.lonza.com

U.S. Scientific Support: 800-521-0390
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EU Scientific Support: 32 (0) 87 321 611
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Cat. no.	Product	Format	Size
BE02-044Q	PowerFeed™ A	Liquid	1 L
BE15-044D	PowerFeed™ A Kit*	Powder	10 L
BE15-044F	PowerFeed™ A Kit*	Powder	50 L
BE15-044J	PowerFeed™ A Kit*	Powder	100 L
BE15-044Q	PowerFeed™ A Kit*	Powder	200 L
BE15-044L	PowerFeed™ A Kit*	Powder	500 L

*Contains: basal powder and ferric citrate

XIII. 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	

XIV. Product use statement

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

****Products are for Research Use Only**

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