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PowerCHO™ Serum-free Medium, Chemically Defined

Instructions for use

Contents:

I. Introduction

PowerCHO™ 1, 2 and Advanced Media and are chemically defined, serum-free, protein-free, non-animal origin media designed to support the growth of CHO cell lines. These media can support growth of CHO K1, DXB11, and DG44. They are free of hydrolysate, but contain a low amount of recombinant human insulin and support high density growth with superior longevity and production. Downstream processing is simplified, as the only proteins present in the supernatant are produced by the cells themselves.

The PowerCHO™ Media are free of glutamine, hypoxanthine, thymidine and phenol red.

The PowerCHO™ Media are available in 1L bottle liquid formulations and various powder kit configurations. Custom productions are also possible, depending on need.

For answers to frequently asked questions regarding these products, please visit our FAQ Database: http://knowledge.lonza.com

II. Unpacking and storage instructions

1. Check all containers for leakage or breakage.
2. Store PowerCHO™ Media protected from light at 2 - 8°C.

III. Preparation of media

1. PowerCHO™ Media is prepared without L-glutamine. For CHO cell lines requiring the addition of glutamine, we recommend adding 4 - 8 mM L-glutamine or UltraGlutamine at the start of the culture.
2. Store supplemented PowerCHO™ Media protected from light at 2 - 8°C.

IV. Thawing of cells/initiation of culture

Initiate cell cultures according to your specific protocol or follow the instructions below. If the culture is initiated from cryopreserved cells, we recommend that cells be thawed into your current control medium. After recovery from thaw, cells may be seeded directly into PowerCHO™ Medium. Cell growth and/or viability may decrease slightly for 1-2 passages during adaptation however, most cells should readily adapt to PowerCHO™ Medium.

1. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure and then retighten. Quickly thaw the cryovial in a 37°C water bath, being careful not to submerge the entire vial. Watch your cryovial closely; when the last sliver of ice melts, remove it.

NOTE: Thawing the cells for longer than 2 minutes may result in less than optimal results.

2. Using a micropipette, gently add the thawed cell suspension to a 50 mL centrifuge tube containing 5 -10 mL of room temperature control medium or PowerCHO™ Medium.
3. Centrifuge the cell suspension at 200 x g for 5 minutes at room temperature. Aspirate the supernatant and resuspend the cell pellet with 2-5 mL of control medium or PowerCHO™ Medium.
4. Gently mix the cells by pipetting up and down. Count the cells with a hemacytometer or cell counter and calculate the viable cell density.
5. Use the following equation to calculate the volume of cell suspension to seed into your vessels. The recommended seeding density for CHO cells at thaw is 2.5 to 3.0 x 10^5 cells/mL.

$$\text{Seeding volume} = \frac{250{,}000 \text{ cells/mL} \times 30 \text{ mL}}{\#\text{ viable cells/mL}}$$

6. Add "seeding volume" and media (30 mL minus seeding volume) to a total volume of 30 mL.
7. Plate cells into appropriate culture vessel and place on a shake platform at 80 - 120 rpm in a humidified 37˚ C incubator.
8. Incubate for 48 - 72 hours.
9. Perform a viable cell count.
10. Passage your culture for a minimum of two times to allow for recovery from thaw and adaptation to PowerCHO™ Medium prior to experiment setup.

V. Initial evaluation of PowerCHO™ Medium in shakers

Prior to experiment setup, cells should be ≥85% viable and should have been passaged a minimum of two to three times in PowerCHO™ Medium (and your control medium for comparison).

NOTE: For the initial feed experiment, we recommend 125 mL shake flasks with an initial volume of 25 - 35 mL.

1. Add 25 - 35 mL PowerCHO™ Medium to two shake flasks, and add 25 - 35 mL control medium to a second set of two shake flasks.
2. Place the flasks in a 37°C incubator to equilibrate while the cell suspensions are being prepared.
3. Count the viable cells in the stock cultures grown in PowerCHO™ Medium and control medium.
4. The recommended seeding density is 2 x 10^5 cells/mL. Prepare 4 x 200,000 cells/mL x 25 mL = 2 x 10^7 cells total for each set (PowerCHO™ Medium and control medium) of the duplicate shakers:

$$\frac{2 \times 10^7 \text{ cells}}{\text{Viable stock cell count}} = \text{Volume of stock needed (for 25 mL culture)}$$

5. Centrifuge calculated stock volume at 250 x g for 5 minutes at room temperature. Aspirate supernatant and resuspend cells in 4mL PowerCHO™ Medium or control medium.
6. Add 1 mL resuspended cells to each duplicate shaker, first for the two PowerCHO™ Media shakers, then for the two control shakers.
7. Set parameters as required by your particular cell line: temperature, agitation rate (rpm).
8. Count and record the viable cells daily from approximately Day 5 until viability drops below 50%, then discard.
9. Samples should be taken for productivity measurement near peak density and daily until flasks are discarded.

VI. Additional information

Other procedures normally used for the particular cell line (for example: use of sodium butyrate) may be conducted on the normal, appropriate schedule.

VII. Ordering information

Cat. no.	Product	Size
BE12-927Q	PowerCHO™ Advance Medium	1 L
BP12-770Q	PowerCHO™ 1 Serum-free Medium	1 L
BP12-771Q BELN12-771Q	PowerCHO™ 2 Serum-free Medium	1 L
12-771P20 BE12-771P20	PowerCHO™ 2 Serum-free Medium	20 L

Related products:

Cat. no.	Product	Size
17-605E* BE17-605E* BEBP17-605E	L-Glutamine (200 mM)	100 mL
BE17-605E/U1*	UltraGlutamine I	100 mL
BEBP12-029Q	ProCHO™ 4 Serum-free Medium	1 L

BP12-766Q BELN12-766Q	ProCHO™ 5 Serum-free Medium	1 L
15-613I	Sodium Bicarbonate Powder	500 g
17-613E* BE17-613E*	Sodium Bicarbonate 7.5% Solution	100 mL
BEBP17-855E	ProHT Supplement (100x)	100 mL
BP12-769E	ProFreeze™ NAO 2x	100 mL
15-770F* BE15-770F*	PowerCHO™ 1 Chemically Defined, Serum-free Medium, Powder	50 L
BE15-771ND	PowerCHO™ 2 Chemically Defined, Serum-free Medium, Powder	10 L
BE15-771NF	PowerCHO™ 2 Chemically Defined, Serum-free Medium, Powder	50 L
BE17-771NJ	PowerCHO™ 2 Chemically Defined, Serum-free Medium, Powder	100 L
15-1027J BE15-1027J	PowerCHO™ Advance Chemically Defined, Serum-free Medium, Powder	100 L
15-1027D BE15-1027D	PowerCHO™ Advance Chemically Defined, Serum-free Medium, Powder	10 L

Product use statements

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

***THESE PRODUCTS ARE FOR RESEARCH USE ONLY**. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or in vivo procedures.