

ProCHO™ Chemically Defined, Serum-free CHO Medium Instructions for use

Contents:

Section	Description	Page
I	Introduction	1
II	Unpacking and storage instructions	1
III	Preparation of media	1
IV	Thawing of cells/initiation of culture	1
V	Initial evaluation of ProCHO™ Medium in shakers	2
VI	Additional information	2
VII	Ordering information	2

I. Introduction

ProCHO™ 4 and 5 Serum-Free Media and are the first generation in CHO media, optimized for cell growth and protein production. The ProCHO™ Series are serum-free, protein-free and non-animal origin media formulated to support high-density CHO cells in suspension. For therapeutic bioprocessing applications, these protein-free formulations also facilitate both downstream purification and regulatory compliance.

ProCHO™ Media are made with Pluronic® F-68 and without L-glutamine or nucleosides (hypoxanthine and thymidine).

The ProCHO™ 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 and citations regarding these products, please visit our knowledge center: <https://knowledge.lonza.com>

II. Unpacking and storage instructions

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

III. Preparation of media

1. ProCHO™ 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 ProCHO™ Medium. Cell growth and/or viability may decrease slightly for 1-2 passages during adaptation however, most cells should readily adapt to ProCHO™ 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.

- 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 ProCHO™ Medium.
- 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.
- Gently mix the cells by pipetting up and down. Count the cells with a hemacytometer or cell counter and calculate the viable cell density.
- 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 10⁵ cells/mL.

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

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

V. Initial Evaluation of ProCHO™ 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.

- Add 25-35 mL ProCHO™ Medium to two shake flasks, and add 25-35 mL control medium to a second set of two shake flasks.
- Place the flasks in a 37°C incubator to equilibrate while the cell suspensions are being prepared.
- Count the viable cells in the stock cultures grown in ProCHO™ Medium and control medium.
- The recommended seeding density is 2 x 10⁵ cells/mL. Prepare 4 x 200,000 cells/mL x 25 mL = 2 x 10⁷ cells total for each set (ProCHO™ Medium and control medium) of the duplicate shakers:

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

- 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.
- Add 1 mL resuspended cells to each duplicate shaker, first for the two ProCHO™ shakers, then for the two control shakers.
- Set parameters as required by your particular cell line: temperature, agitation rate (rpm).
- Count and record the viable cells daily from approximately Day 5 until viability drops below 50%, then discard.
- 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
BEBP12-029Q	ProCHO™ 4 Protein-Free Medium with phenol red and Pluronic®, w/o L-gln, hypoxanthine and thymidine	1 L
BP04-919Q	ProCHO™ 4 Protein-Free Medium with Pluronic®, w/o L-gln, phenol red, hypoxanthine and thymidine	1 L
BP12-766Q	ProCHO™ 5 Protein-Free Medium with Pluronic®, w/o L-gln, phenol red,	1 L
BELN12-766Q (EU Only)		

	hypoxanthine and thymidine	
BE12-766P10**	ProCHO™ 5 Protein-Free Medium with Pluronic®, w/o L-gln, phenol red, hypoxanthine and thymidine	10 L bag
BE12-766P20**	ProCHO™ 5 Protein-Free Medium with Pluronic®, w/o L-gln, phenol red, hypoxanthine and thymidine	20 L bag

** Made to order with a minimum of 5

Related products:

Cat. no.	Product	Size
BE15-766D	ProCHO™ 5 Powder Kit	10 L
BE15-766F	ProCHO™ 5 Powder Kit	50 L
BE15-766L	ProCHO™ 5 Powder Kit	500 L
(BE)17-605E* BEBP17-605E	L-Glutamine (200 mM)	100 mL
BE17-605E/U1*	UltraGlutamine I	100 mL
BE04-684E*	UltraGlutamine II	100 mL
BP12-770Q	PowerCHO™-1 Serum-free Medium	1 L
BP12-771Q BELN12-771Q (EU Only)	PowerCHO™-2 Serum-free Medium	1 L
BE12-927Q	PowerCHO™ Advance Serum-free Medium	1 L
BEBP17-855E	ProHT™ Supplement (100x)	100 mL
BP12-769E	ProFreeze™ NAO 2x	100 mL

Product use statement

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.

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Pluronic® acid is a registered trademark of BASF corporation.