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TheraPRO® CHO Media System

Instructions for use

It is important to follow instructions provided in this document for the successful use of TheraPRO® CHO Media System. If customers are unsure of any steps, then we recommend that they get in touch with Lonza Bioscience Media Technical Support.

Lonza GS Gene Expression System® License Holders should refer to the GS® Manual Portal for tailored instructions.

I. Introduction

TheraPRO® CHO Media System is optimized to support the culture of stable GS-CHO cell lines to maximize the production of large quantities of high-quality recombinant proteins. The media system is associated with high product quality across culture scales from cell line development through bioprocess manufacturing.

TheraPRO® CHO Media System is manufactured in compliance with cGMP and all components are chemically-defined and non-animal origin (NAO).

TheraPRO® CHO Media System is primarily designed and optimized for use with fed-batch systems only.

II. Storage

TheraPRO® CHO Media System should be stored at 2°C to 8°C, protected from light. For storage instructions specific to production medium and feed, refer to section III (Instructions for use).

III. Instructions for use

Media and feed preparation

TheraPRO® CHO Production Medium

1. Measure 1000 g of deionized water at a temperature of 18°C to 25°C.
2. Add 25.3 g of TheraPRO® CHO Production Medium to water.
3. Gently mix for 90 minutes. Do not vortex. Confirm that solution is completely dissolved and keep vessels protected from light throughout the mixing process.
4. Add 60 microliters of Monoethanolamine, ‘Baker Analyzed’, in 1 liter of TheraPRO® CHO Production Medium to further improve cell culture performance.
5. To measure the pH and osmolality transfer media to an appropriate lidded tube. The pH should be between 6.81 to 7.19. Higher mixing times can lead to an increase in pH. The osmolality should be between 288 to 310 mOsm.
6. Sterile filter through a 0.22 micron filter membrane (PES) within 45 minutes of obtaining pH and osmolality measurements. Filtering as soon as possible will help prevent accumulation of bioburden.
7. Store in a suitable bag at 2°C to 8°C to limit head-space for maximum media stability, and protect from light, for up to 21 days, or up to 2 days maximum when filled in bioreactor vessel with temperature between 21°C to 37°C.

TheraPRO® CHO Feed

1. Measure 879 g of deionized water at a temperature of 33°C to 37°C (target: 35°C).
2. Add 117.09 g of TheraPRO® CHO Feed to water (Target temp: 35°C).
3. Gently mix for 90 minutes at ambient temperature. Do not vortex. Confirm that the solution is completely dissolved. There is no need to continuously heat the water during mixing. Keep the vessels protected from light throughout the process.
4. Measure the pH and osmolality using an appropriate lidded tube. The pH should be between 5.52 to 5.90. The osmolality should be between 905 – 1029 mOsm/Kg.
5. Sterile filter through a 0.22 micron filter membrane (PES within 45 minutes of obtaining pH and osmolality measurements. Filtering as soon as possible will help prevent accumulation of bioburden.
6. Store in suitable bags to limit head-space for maximum feed stability, and protect from light, for up to 20 days from the date of preparation at ambient temperature (18°C to 25°C). While the above practice is strongly recommended, it's possible that feed storage in bags may only be feasible for bench top bioreactors and higher scales. For shake flasks and micro-bioreactors, we recommend that feed, once prepared, is aliquoted and stored in centrifuge tubes filled all the way to the top to limit head space. The tubes can then be stored in ambient temperature 18°C to 25°C for up to 20 days. It should be noted that the aliquots are meant for single-use only. Once an aliquot is opened and used, it should be discarded.

D-Glucose Feed (400g/L)

1. Measure 747 g of deionised water at a temperature of 42°C to 45°C into a sterile vessel containing a stir bar.
2. While stirring the water, slowly add 400 g of D-glucose to the vessel.
3. Vigorously mix for a minimum of 30 minutes. Confirm that solution is completely dissolved.
4. Measure the temperature. The temperature must be < 37°C before filtration.
5. Sterile filter through a 0.22 micron PES filter membrane within 6 hours of adding the powdered D-glucose to the water.
6. Store at 18°C to 24°C for up to 28 days in a sterile vessel.

Warming media and additives

1. Media used for subculturing and fed-batch cultures should be pre-warmed in a water bath for 1 to 6 hours to 37°C before use. Discard any unused warmed media.
2. All supplements (e.g. L-glutamine) should be added to the medium on the day of use unless otherwise stated.
3. Supplements should be added to pre-warmed media and mixed thoroughly. Frozen supplements should be pre-warmed in a water bath for 1 to 6 hours to 37°C before use. Some agitation may be required to ensure complete dissolution upon thawing, particularly with L-glutamine.

Table 1. Supplementation guide

Cell type	Process step	Medium	Supplement concentration
GS Knockout CHO-K1	Host cell line	TheraPRO® CHO Expansion Medium	6mM L-glutamine
CHOK1			
GS Knockout CHO-K1	Pools	TheraPRO® CHO Expansion Medium	50 µM MSX
CHOK1			25 µM MSX
GS Knockout CHO-K1	Clonal cell lines	TheraPRO® CHO Expansion Medium	
CHOK1			

NOTE: For transfectant cell lines derived from the CHO-K1 GS-Knockout host cells, L-glutamine should not be used.

Revival and subculture procedure

We strongly recommend that the cell lines that were previously cryo-preserved in non-Lonza media be systematically adapted to TheraPRO® CHO Expansion Medium. This requires steps using TheraPRO® CHO Expansion Medium and other media systems used for cryo-preservation/ previous sub-cultures, 10:90, 30:70, 50:50, 70:30, 90:10, 100%, respectively. Cultures should be kept in each media ratio for 1 – 2 passages or until cells reach >90% viability and 3 – 4E6 cells/mL viable cell concentration after 4 days of sub-culturing when seeded at 0.2 – 0.7 E6 cells/mL.

1. Rapidly thaw a vial of frozen cells by placing in a sterile container with pre-warmed water to 30°C to 37°C, without submerging the cap.
2. Transfer the thawed cell suspension into a 50 mL centrifuge tube.
3. Slowly add 10 mL of the appropriate growth medium to the tube by cascading the medium down the side of the tube for about 1 minute.
4. Slowly add another 33.5 mL of growth medium to the tube, again by cascading the medium down the side of the tube for about 1 minute.
5. Centrifuge the cell suspension at 200 x g for 5 minutes.
6. Discard the supernatant. Resuspend the cell pellet by gentle pipetting with 10 mL of growth medium until the suspension is homogenous.
7. Determine viable cell density using an automated device or manual counting method.
8. Calculate the volume of cell culture necessary to seed at a concentration of 0.3 x 10E6 viable cells/mL to 0.7 x 10E6 viable cells/mL.
9. Transfer the volume of cell culture and growth medium to the corresponding vessel.
    a. If using sealed caps, purge the headspace prior to sealing with 5% CO_2 for the time indicated in Table 2. Incubate on an orbital shaker set to 35.5°C to 37°C and 135 to 145 rpm.
    b. If using vented caps, incubate on an orbital shaker set to 35.5 to 37°C and 135 to 145 rpm, >85% relative humidity and 5% CO_2 in air.

Table 2. Cell culture flask condition recommendations

Vessel	Culture volume (mL)	Purge time with 5% CO_2 in air (sec)*
25 mL shake flask	1 – 7.5	5
125 mL shake flask	5 – 30	10
250 mL shake flask	30 – 50	10
500 mL shake flask	50 – 100	20
1000 mL shake flask	100 – 200	20
1 L roller bottle**	100 – 200	20
2 L roller bottle**	200 – 400	30

*The pressure of the CO_2 in air gas line should be set to 4 to 6 psi.

**Roller bottles are used in shaking mode of 135 to 145 rpm.

10. On day 3, perform the first subculture to ensure that the cell density is 1.5 – 5.0 x 10E6 viable cells/mL with >95% viability. Re-seed the cells into TheraPRO® CHO Expansion Medium, with the corresponding supplementation from Table 1, at a concentration of 0.2 x 10E6 viable cells/mL to 0.7 x 10E6 viable cells/mL, subculturing every 3 to 4 days afterward.

Cloning procedure

1. Transition the cells into the recommended pre-warmed cell culture medium as shown in Table 3 during the cloning method of choice.

Table 3. Cloning medium recommendations by cloning method

Cloning method	Cloning medium
Limiting Dilution	TheraPRO® CHO Cloning Medium (preferred)
FACS and Single Cell Printing	TheraPRO® CHO Cloning Medium
Microfluidic /Beacon Based Systems	TheraPRO® CHO Expansion Medium + 10 mM HEPES must be added immediately before cloning

2. For limiting dilution and FACS sorting, use cloning medium supplemented with rhAlbumin at a concentration of 1 to 6 g/L). For limiting dilution resuspend the counted number of cells into the supplemented cloning medium such that when the desired volume of medium containing the cells is added to the wells of a 96-well plate, the probability of having a

cell/well<1.0. FACS sorting: use a FACS sorter to deposit one cell/well in 96 well plates already containing the desired volume of supplemented cloning medium. Incubate the plates at 37°C and 10% CO_2.

3. Check the plates periodically for appearance of single CHO cell colonies. Growing single colonies can be moved to 24 well plates and incubated further until ready for further expansion. Consider adding Phenol red as an indicator for cell growth.
4. When using the Beacon, ensure you follow the vendor's recommendations and cover the bottle with a silicone cap to help prevent pH changes. Add anti-clumping agent to the medium as required.
5. Upon completion of the cloning step, transition the cells back into TheraPRO® CHO Expansion Medium, in preparation of the fed-batch procedure.

Cryopreservation procedure

1. Determine the viable cell density and calculate the volume of medium required to seed the desired number of vials at 1.5 x 10E7 viable cells/vial.
2. On day 3 or 4 of subculture, harvest the cells in the mid-log phase, ensuring viability of > 80%.
3. On the day of cryopreservation, prepare the required volume of cryopreservation medium consisting of 7.5% DMSO and 92.5% TheraPRO® CHO Expansion Medium, without additional supplements.

NOTE: Add the DMSO to the TheraPRO® CHO Expansion Medium rather than adding TheraPRO® CHO Expansion Medium to the DMSO. Ensure the solution is well-mixed.

4. Harvest the cells by centrifuging the cell suspension at 200 x g for 5 minutes.
5. Resuspend the cell pellet in the pre-determined volume of cryopreservation medium.
6. Aliquot the cell suspension into cryovials and place the vials into a suitable cryopreservation device, using a programmable controlled-rate cell freezer.
7. Transfer frozen vials to a vapor phase liquid nitrogen storage container as soon as cryopreservation is complete.

Instruction for fed-batch shake flask culture

1. Once the cells have undergone 2 – 3 passages in TheraPRO® CHO Expansion Medium, they should be transitioned into TheraPRO® CHO Production Medium.
2. Once the cells have undergone 2 – 3 passages in TheraPRO® CHO Expansion Medium, they should be transitioned into TheraPRO® CHO Production Medium for a fed-batch run.
3. For this determine viable cell density of the culture using an automated device or manual counting method.
4. Calculate the volume of cell suspension necessary to seed at a concentration of 0.4 x 10E6 viable cells/mL to 0.8 x10E6 viable cells/mL.
5. Transfer the required volume of pre-warmed TheraPRO® CHO Production Medium to a new culture vessel, using Table 4 as a guide.

Table 4. Fed-batch shake flask volume recommendations

Vessel	Culture volume (mL)
500 mL shake flask	50 – 100
1000 mL shake flask	100 – 200
2000 mL shake flask	200 – 400

6. Refit the cap and place the new culture onto an orbital shaker incubator set to 37°C, 135 to 145 rpm, 5 to 8% CO_2 in air and > 85% relative humidity. Flasks should be selected to have sufficient venting and fill volume to support a viable concentration of up to 40 x 10e6 viable cells/mL.
7. On day 1, determine the viable cell density of the culture for optimal feed addition. The TheraPRO® CHO Feed is added daily from day 1 onward. If antifoam is required, it may be added as needed. Use Table 5 and 6 as guides for typical feed ranges, depending on cell type.

NOTE: We strongly recommend that customers follow feeding regime as suggested in table 5 and 6. Not doing so may result in hampered cell growth and reduced viability and titers. However some optimization may be required.

8. Between day 6 to day 8, change the temperature of the incubator to 33°C and maintain until harvest.
9. Harvest the cultures on day 15.

Table 5. TheraPRO® CHO Feeding Regime for GS CHO cells

Optimization may be required to determine the best feed concentration.

VCD Range (x $10E^6$ cells/mL)	% Volume of TheraPRO® CHO Feed per/L for CHO-K1 cells	% Volume of TheraPRO® CHO Feed per/L for GS KO CHO-K1 cells
0 to 5.99	0.8% to 1.2%	1% to 1.5%
6.00 to 11.99	1.3% to 2%	1.6% to 2.5%
12.00 to 17.99	2.1 % to 2.7%	2.5% to 3.4%
18.00 to 22.00	2.8% to 3.1%	3.5% to 3.9%
>22.00	3.2%	4%

Table 6. Fed-batch culture schedule in TheraPRO® CHO Media System

	Day																
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Revival in TheraPRO® CHO Expansion Medium																	
Inoculation into TheraPRO® CHO Production Medium																	
Addition of TheraPRO® CHO Feed (daily)																	
Supplementation of D-glucose, check on daily basis to maintain above 3g/L at all times																	
Apply the temperature shift to 33°C on day 6 (no later than day 8) and maintain 33°C for the remainder of the process																	
Optional: Addition of antifoam																	
Harvest																	

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V. Product use statement

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