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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 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 (Instruction 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. Mix for 90 minutes. Do not vortex. Confirm that solution is completely dissolved.
4. Optional: add 60 microliters of Monoethanolamine, 'Baker Analyzed', in 1 liter of TheraPRO CHO Production Medium to further improve cell culture performance.
5. Measure the pH and osmolality. The pH should be between 6.9 to 7.2. The osmolality should be between 290 to 305 mOsm.
6. Sterile filter through a 0.2 micron filter membrane (PES or PVDF) within 45 minutes of obtaining pH and osmolality measurements. Filtering as soon as possible will help prevent accumulation of bioburden..
7. Store in bags (i.e., Flexboy or FlexSafe) 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

8. Measure 879 g of deionized water at a temperature of 33°C to 37°C (target: 35°C).
9. Add 117.09 g of TheraPRO® CHO Feed to water (Target temp: 35°C).
10. Mix 90 minutes at ambient temperature. Do not vortex. Confirm that solution is completely dissolved.
11. Measure the pH and osmolality. The pH should be between 5.5 to 5.9. The osmolality should be between 950 to 1030 mOsm.
12. Sterile filter through a 0.22 micron filter membrane (PES or PVDF) within 45 minutes of obtaining pH and osmolality measurements.

Filtering as soon as possible will help prevent accumulation of bioburden.

13. Store in bags (i.e., Flexboy or FlexSafe) to limit head-space for maximum feed stability, and protect from light, for up to 21 days from the date of preparation. 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 be stored in 2°C to 8°C for up to 21 days. It should be noted that the aliquots are meant for a single only. Once an aliquot is opened and used, it should be discarded.

D-Glucose Feed (400g/L)

14. Measure 747 g of water for injection (WFI) at a temperature of 42°C to 45°C into a sterile vessel containing a stir bar.
15. While stirring the water, slowly add 400 g of D-glucose to the vessel.
16. Vigorously mix for a minimum of 30 minutes. Confirm that solution is completely dissolved.
17. Measure the temperature. The temperature must be < 37°C before filtration.
18. Sterile filter through a 0.2 micron filter membrane within 6 hours of adding the powdered D-glucose to the water.
19. Store at 18°C to 24°C for up to 28 days in a sterile vessel.

Warming media and additives

20. 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.
21. All supplements (e.g. L-glutamine) should be added to the medium on the day of use unless otherwise stated.
22. 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
CHO-K1	Host cell line	TheraPRO® CHO Expansion Medium	6mM L-glutamine
GS Knockout CHO-K1	Pools	TheraPRO® CHO Expansion Medium	50 µM MSX
GS Knockout CHO-K1	Clonal cell lines	TheraPRO® CHO Expansion Medium	

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 should be systematically adapted to TheraPRO® Expansion Medium. This requires steps using TheraPRO® Expansion Medium and other media system 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 3-4 million cells/mL viable cell concentration after 4 days of sub-culturing.

23. 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.
24. Transfer the thawed cell suspension into a 50 mL centrifuge tube.
25. 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.
26. 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.
27. Centrifuge the cell suspension at 200 x g for 5 minutes.
28. Discard the supernatant. Resuspend the cell pellet by gentle pipetting with 10 mL of growth medium until the suspension is homogenous.

29. Determine viable cell density using an automated device or manual counting method.
30. Calculate the volume of cell culture necessary to seed at a concentration of 0.3 x 10e6 viable cells/mL to 0.7 x10e6 viable cells/mL.
31. 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.

32. 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 x10e6 viable cells/mL, subculturing every 3 to 4 days afterward.

Cloning Procedure

33. 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 Expansion Medium
FACS and Single Cell Printing	TheraPRO® CHO Cloning Medium
Microfluidic/Beacon-Based Systems	TheraPRO® CHO Expansion Medium + 10 mM HEPES added min one subculture before cloning

34. Upon completion of the cloning step, transition the cells back into TheraPRO® CHO Expansion Medium, in preparation of the fed-batch procedure.

Cryopreservation Procedure

35. 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.
36. On day 3 or 4 of subculture, harvest the cells in the mid-log phase, ensuring viability of >80%.
37. 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.
38. Harvest the cells by centrifuging the cell suspension at 200 x g for 5 minutes.
39. Resuspend the cell pellet in the pre-determined volume of cryopreservation medium.
40. Aliquot the cell suspension into cryovials and place the vials into a suitable cryopreservation device, using a programmable controlled-rate cell freezer.
41. Transfer frozen vials to a vapor phase liquid nitrogen storage container as soon as cryopreservation is complete.

Instruction for Fed-Batch Shake Flask Culture

42. Once the cells have been sub-cultured 2 to 3 times in TheraPRO® CHO Expansion Medium, the cells can be transitioned into the TheraPRO® CHO Production Medium. This is considered day 0.
43. Determine viable cell density of the culture using an automated device or manual counting method.
44. 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.
45. 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

46. Refit the cap and place the new culture onto an orbital shaker incubator set to 37°C, 135 to 145 rpm, 7 - 9% 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.
47. 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, maintaining >1g/L D-Glucose in the culture. If antifoam is required, it may be added as needed. Use Table 5 as a guide for typical feed ranges, depending on cell type. Optimization may be required to determine the best feed concentration.

Table 5. Feeding Guidelines

Viable Cell Density Range (10e6 viable cells/mL)	% Volume of TheraPRO® CHO Feed per/L for CHO-K1 Cells	% Volume of TheraPRO® CHO Feed/L for GS Knockout CHO-K1 Cells
0-11.99	1-2%	1-2.5%
>12	2-3%	2.6-4%

We strongly recommend that customers follow feeding regime as suggested above. Not doing so may result in hampered cell growth and reduced viability and titers.

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

Fed-Batch Shake Flask 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																	
Temperature change to 33°C																	
Addition of TheraPRO® CHO Feed daily																	
Supplementation of D-glucose, check on daily basis to maintain at >1 g/L																	
Optional: Addition of antifoam																	
Harvest																	

Product use statement

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