Version 2，2023

The User's Manual—CHO-1 CDM08

Basic Information Introduction

Product Introduction

CHO-1 CDM08 is chemically-defined (CD) basal media，without protein, hydrolysate, growth factor and any animal-derived components, it suitable for high-density cell suspension expansion and stable expression of CHO cell lines(CHO-K1 and ect), it can effectively support CHO high-level expression of recombinant proteins and antibodies.CHO-1 CDM08 combine with commercial feed media, can support high-density cell expansion, viability maintenance and expression, achieving high-level and high-quality protein/antibody titer.

Application Scope

CHO-1 CDM08 can be used in the process of CHO cell line cell thawing, cell passage, high-density cell suspension culture expansion and stable expression. It can be used in the research and further manufacturing of protein/antibody related products, but cannot be directly used in the human body or used as a medication.

Shipping, Storage and Validity Period

Product	Catalog No.	Storage	Shipping	Validity Period
CHO-1 CDM08	LQ08, Liquid	2℃ ~ 8℃, Protect from light	2℃ ~ 8℃, Protect from light	12 months
CHO-1 CDM08	DP08, Powder	2℃ ~ 8℃, Protect from light	2℃ ~ 8℃, Protect from light	24 months

Protocol for Hydration of Powder Medium

1. Fill the mixing container with purified water (20 ~ 30℃) at 80% of the final volume.
2. Slowly add 22.90 g/L of powder medium with gentle stirring. Mix for 30 minutes.
3. Adjust the pH to 5.0 ~ 6.0 using 1M ~ 5M NaOH solution.
4. Slowly add 2.10 g/L $NaHCO_3$ to the solution with gentle stirring, mix for 5 ~ 10 minutes.
5. Adjust the pH to 6.8 ~ 7.2 using 5M NaOH or 1M HCl solution, mix for 10 minutes.

6. Adjust the solution volume to 100% with purified water, mix for 10 minutes.

7. Filter immediately the media with a 0.22 μm membrane filter.

Quality Index of Dry Powder and Liquid Media

Product Index	CHO-1 CDM08 (LQ08), Liquid	CHO-1 CDM08 (DP08), Powder
Appearance	Brown, clear liquid	Off white powder
pH	6.8 ~ 7.4	6.8 ~ 7.2 (pre-filter)
Osmolality (mOsmol/kg)	260 ~ 320	270 ~ 320
Solubility	--	Dissolve well according to the protocol for hydration of powder medium
Endotoxin (EU/mL)	< 3	< 3
Sterility	Negative	--
Bioburden	--	Aerobic bacteria: < 200 CFU/g Molds and yeasts: < 50 CFU/g

Reference Cell Culture Protocol

Culture Conditions

Parameter		Value
Culture volume	50 mL TPP Tube	10 ~ 30 mL
	125 mL Shake flask	15 ~ 40 mL
	250 mL Shake flask	40 ~ 80 mL
	500 mL Shake flask	100 ~ 200 mL
	1000 mL Shake flask	200 ~ 300 mL
Shaking speed	TPP Tube	50mm amplitude：200 rpm
	Shake flask	25mm amplitude：150 rpm
	Shake flask	50mm amplitude：90 ~ 120 rpm
Culture environment	Seeding density	0.5×10^6 cells/mL
	Incubation temperature	37℃
	Incubation CO_2 concentration	5%
	Incubation relative humidity	> 80% RH

Cell Thawing

1. Pre-warm the medium (CHO-1 CDM08) in 37°C water bath.

2. Spray the outside of the medium bottle with 75% alcohol and place the bottle into the bio-safety cabinet.

3. Thaw one vial at a time in 37°C water bath. Gently agitate the vial within 1 minutes until the ice in the vial melting.

4. Pipet the contents from the vial gently into a centrifuge tube containing 30mL of pre-warmed medium (CHO-1 CDM08).

5. Centrifuge 150 g ~ 300 g for 5 minutes. Discard the supernatant and re-suspend cells in 10 ~ 30mL fresh pre-warmed medium (CHO-1 CDM08), then adjust the cell density to 0.5×10^6 cells/mL.

6. Sample 0.5mL of cell suspension and analyze the viable cell density ($\times10^6$ cells/mL) and viability (%) of the sample using cell counter.

7. If the cell viability > 85%, incubate cells in the specified condition (refer to "culture conditions" table).

Cell Passage

1. Pre-warm the medium (CHO-1 CDM08) in 37°C water bath for 20 ~ 30 min.

2. Cells with viable cell density ≥ 2×10^6 cells/mL, cell viability ≥ 85%, and in the middle of logarithmic growth phase were selected for passage.

3. According to the seed cell density of 0.5×10^6 cells/mL, calculate the amount of total number of seed cells.

4. Seed cells at 0.5×10^6 cells/mL in the flask and add a certain volume of pre-warmed fresh medium.

5. Incubate cells in the specified environment condition (refer to "culture conditions" table).

6. Passage cells with fresh CHO-1 CDM08 medium according to the above steps once every 2 ~ 3 days.

7. If the viable cell density is less than 2.0×10^6 cells/mL or the viability is lower than 85% before passaging, the cells need to be centrifuged at 150 g ~ 300 g for 5 minutes. Carefully remove the spent media, then resuspend cells with preheated CHO-1 CDM08 medium, passage cells after sampling and counting.

Adaptation

Direct Adaptation

1. For cells can direct adapt, transfer cells suspension cultures into CHO-1 CDM08 directly, and the seed cell density refer to the cell passage procedure.

2. Cell passage until cell expression steadily.

3. When VCD reaches 2×10^6 cells/mL and ≥ 90% viability（3 ~ 4 days）. At this point, the cells had been successful

adapted.

Sequential Adaptation

For cells growing in 5 ~ 10% serum or SFM media. Sequential adaptation should be performed.

1. Seed cells at 0.5×10^6 cells/mL in original cell culture media.

2. Sample and cell count every day until the VCD reaches 2×10^6 cells/mL.

3. Seed cell density at 0.5×10^6 cells/mL, subculture cells into stepwise increasing ratios of complete CHO-1 CDM08 medium to original medium with each subsequent passage (25:75, 50:50, 75:25, 90:10, 100:0).

4. When VCD reaches 2×10^6 cells/mL and ≥ 90% viability（3 ~ 4 days）. At this point, the cells had fully adapted to CHO-1 CDM08 media.

Cell Cryopreservation

1. Prepare cells, harvesting in mid-log phase of growth with viability > 90%.

2. Sample and cell counting, calculate the required volume of cell freeze solution to give a final density of 1×10^7 cells/mL.

3. Prepare the cell freeze solution: 90% CHO-1 CDM08 + 10% DMSO, store at 4°C.

4. Centrifuge 300 g for 5 minutes, discard the supernatant and re-suspend cells with the cell freeze solution.

5. Immediately dispense aliquots of cells suspension into cryovials according to the specific needs of the project.

6. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).

7. Transfer to liquid nitrogen tank for storage.

Reference Fed Feeding Strategy

Fed Feeding Strategy

1. Seed cells at 0.5×10^6 cells/mL into CHO-1 CDM08.

2. Starting from day D3, fed feeding according to the following feeding strategy:

Feeds/Time	D3	D5	D7	D9	D11	D13
Tobitec FA / FB	2/0.2%	2/0.2%	2/0.2%	2/0.2%	2/0.2%	2/0.2%
Tobitec FA / FB	3/0.3%	3/0.3%	3/0.3%	3/0.3%	3/0.3%	3/0.3%

Tobitec FA / FB	4/0.4%	4/0.4%	4/0.4%	4/0.4%	4/0.4%	4/0.4%
Glucose	When the glucose concentration is below 4 g/L, glucose is added at the final concentration of 6 g/L					

Suggestions:

It is suggested that the density of viable cells in the first feeding should be 4 ~ 6 $\times 10^6$ cells/mL, and the feeding can be advanced or delayed according to the growth of cells.

In order to maintain high cell density and high viability during fed batch culture, it is recommended to lower the culture temperature when the viable cell density exceed 15×10^6 cells/mL.