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The User's Manual—CHO CDM10

Basic Information Introduction

Product Introduction

CHO CDM10 is chemically-defined (CD) basal media, without protein, protein hydrolysate and any animal-derived components, it suitable for high-density cell suspension expansion and transient transfection of diffrent CHO cell lines (CHO-K1, CHO DG44, CHO-S and ect.), it can effectively support CHO high-level expression of recombinant proteins and antibodies. CHO CDM10 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 CDM10 can be used in the process of CHO cell line cell thawing, cell passage, high-density cell suspension culture expansion and transient transfection. It can be used in the research and large-scale production of cell culture-based biologics, 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 CDM10	LQ10, Liquid	2℃ ~ 8℃, Protect from light	2℃ ~ 8℃, Protect from light	12 months
CHO CDM10	DP10, 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 (25 ~ 30℃) at 80% of the final volume.

2. Slowly add 21.27 g/L of powder medium with gentle stirring. Mix for 30 minutes.

3. Adjust the pH to 5.0 ~ 6.0 using 5M NaOH solution.

4. Slowly add 1.80 g/L $NaHCO_3$ to the solution with gentle stirring, mix for 5 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 powder and Liquid Media

Product Index	CHO CDM10 (LQ10), Liquid	CHO CDM10 (DP10), Powder
Appearance	Light yellow, clear liquid	Off white powder
pH	6.8 ~ 7.4	6.8 ~ 7.2 (pre-filter)
Osmolality (mOsmol/kg)	260 ~ 320	260 ~ 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 CDM10) 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 30 mL of pre-warmed medium (CHO CDM10).

5. Centrifuge 150 g ~ 300 g for 5 minutes. Discard the supernatant and re-suspend cells in 10 ~ 30mL fresh pre-warmed medium (CHO CDM10), then adjust the cell density to 0.5×10^6 cells/mL.

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

7. If the cell viability > 85%, incubate the shaker flask in the specified condition.

Cell Passage

1. Pre-warm the medium (CHO CDM10) 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 CDM10 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 CDM10 medium, passage cells after sampling and counting.

Adaptation

Direct Adaptation

1. For cells can direct adapt, transfer cells suspension cultures into CHO CDM10 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 CDM10 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 CDM10 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 CDM10 + 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 Transient Transfection and Fed Feeding Strategy

CHO Transient and Expression

1. CHO cells that have completed the adaptive culture can be transfected after 3 passages. Prepare fresh CHO CDM10 medium before transfection. If the cell line is not expi CHO, EDTA should be added at the final concentration of 4 mg/L.

2. Centrifuge the cells to be transfected at 150 g ~ 300 g for 5 minutes and resuspend cells with fresh medium.

3. Sample and cell counting, dilute to 4 ~ 6×10^6 cells/mL with pre-warmed fresh media.

4. To perform transfection operations, the optimal transfection conditions should be optimized for specific

circumstances, and the following transfection conditions are for reference only:

VCD	DNA	PEI
4 ~ 6×10^6 cells/mL, viability > 95%	1.5 mg/L	7.5 mg/L

5. Incubate cells in the specified condition.

Fed Feeding Strategy

Feeds/Time	D1	D4	D7
Tobitec FA/FB	**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		
Glutamine	Control glutamine 2 ~ 6 mmoL/L after transfection or add 4 mmoL/L glutamine according to culture volume during feeding		