Version 2，2025

The User's Manual—CHO Trans kit01

Basic Information Introduction

【Product Name】：CHO Transient Transfection Kit

【Product Model】：CHO Trans kit01

【Product Number】：CHO-TK01

【Packaging Specifications】：

Kit Components	Product Model	Number/Specification	Storage Conditions
CHO Chemical defined medium	CHO CDM10	LQ10-1000×1	2～8℃, protected from light
Two-in-one feed medium	Tobitec FedVigor02	LQ47-125×2	2～8℃, protected from light
Transfection enhancer	CHE STE01	ML701-20×1	2～8℃, protected from light
Transfection reagent (1mg/mL)	Tobitec TR01	ML801-2×4	2～8℃, protected from light

【Product Introduction】：

The CHO Transient kit (CHO Trans kit01) is chemically component-limited and is suitable for high-density culture and transient transfection expression of CHO cells (mainly ExpiCHO-S). The combined use of each group can significantly improve the transfection performance and protein expression level, and has excellent stability and reproducibility. This kit product is suitable for scientific research and the production of large-scale biological products based on cell culture, but it cannot be directly used in the human body or used as a medication.

【Storage Conditions and Validity Period】：

Transport at 2 ~ 8℃, protected from light. Store away from light (for storage temperature, refer to [Packaging Specifications]). The validity period is 6 months.

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【Quality Index】：

Product Index	CHO CDM10 (LQ10), Liquid	Tobitec FedVigor02 (LQ47), Liquid	CHE STE01 (ML701), Liquid	Tobitec TR01 (ML801), Liquid
Appearance	Light yellow, clear liquid	Red, clear liquid	Colourless, clear liquid	Colourless, clear liquid
pH	6.9 ~ 7.5	6.6 ~ 7.4	--	--
Osmolality (mOsmol/kg)	260 ~ 320	240 ~ 340 (5-fold dilutions)	--	--
Endotoxin (EU/mL)	< 3	< 10	< 3	< 3
Sterility	Negative	Negative	Negative	Negative

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	36 ~ 38℃
	Incubation CO_2 concentration	5%
	Incubation relative humidity	> 80% RH

Cell Thawing

1. Pre-warm the medium CHO CDM10 in 37℃ 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℃ water bath. Gently agitate the vial within 1 minutes until the ice in the vial melting.

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4. Pipet the contents from the vial gently into a centrifuge tube containing 30mL of pre-warmed medium CHO CDM10.

5. Centrifuge 150 g ~ 300 g (About 800rpm ~ 1200rpm) 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 0.5mL 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 CDM10 in 37℃ 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. Aseptically transfer the required amount of seed solution and add it to the shake flask containing the required volume of preheated CHO CDM10 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 (About 800rpm ~ 1200rpm) 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 stable growth is achieved.

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.

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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 Three to four days after seeding completely in CHO CDM10 medium, the VCD should reach at least 2×10^6 cells/mL with a cell viability of ≥ 90%. At this point, the cells have fully adapted to the CHO CDM10 medium.

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℃.

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℃ decrease per minute).

7.Transfer to liquid nitrogen tank for storage.

Reference to the Transient Transfection Feeding Strategy

Cell Passage

1. After the CHO cells are fully adapted through the above adaptation procedure, they are subcultured and expanded at a viable cell density of 0.5×10^6 cells/mL until the cell volume meets the requirement for transfection, then proceed to the next step.

2. One day before transfection, dilute the cell density to 2 ~ 3×10^6 cells/mL using fresh CHO CDM10 medium, so that the viable cell density will be 5 ~ 7×10^6 cells/mL at the time of transfection the next day.

Transfection

Taking a 30 mL culture volume as an example (for other culture volumes, convert according to actual needs based on volume), the optimal transfection conditions should be optimized according to specific circumstances. The following transfection conditions are for reference only:

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VCD	DNA	Tobitec TR01
6 ×10^6 cells/mL, viability > 95%	1.5mg/L	7.5mL/L

1. On the day of transfection (D0), first count the cells. Based on the counting results, add fresh medium to adjust the viable cell density to 6×10^6 cells/mL.

2. Dilute and mix 45ug of plasmid DNA with 1.5mL of CHO CDM10 medium, then dilute and mix 225 uL of transfection reagent Tobitec TR01 (1mg/mL) with 1.5mL of CHO CDM10 medium. Add the mixed Tobitec TR01 to the well-mixed DNA solution and gently and slowly mix evenly. Let it stand and incubate for 10 to 15 minutes.

3. Take out the cell fluid to be transfected, and slowly add the DNA and Tobitec TR01 complex to the cell culture medium, shaking the cell fluid well while adding until it is completely added.

4. Place the shake flask under the specified environmental conditions (refer to the "Cell Culture Conditions" in the above table) for culture. Set the shaker temperature to 37℃, record the culture time as D0, and the transfection is completed.

Feeding strategy

1. 8 ~ 24 hours after transfection (D1) : Based on the volume of transfected cells, supplement with 2% CHE STE01 (only once) and 8% Tobitec FedVigor02, and reduce the temperature to 32℃ for cultivation.

2. Subsequently, D4 to D7: Supplement with 8% Tobitec FedVigor02 once every 3 days. When the glucose concentration in the cell culture medium is lower than 4 g/L, add glucose to a final concentration of 6 g/L.

3. The culture can be terminated when the cell viability is ≤ 70%.

【Date of Instruction Manual Approval and Modification】： 2025.08

【Technical Support】：

According to the terms of sales, please contact our technicians with any problems：

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