Version 2，2025

The User's Manual—HEK Trans kit01

Basic Information Introduction

【Product Name】：HEK293 Transient Transfection Kit

【Product Model】：HEK Trans kit01

【Product Number】：HEK-TK01

【Packaging Specifications】：

Kit Components	Product Model	Number/Specification	Storage Conditions
HEK293 Chemical defined medium	HEK293 CDM26 Pro	LQ26-2-1000×1	2 ~ 8℃, away from light
Two-in-one feed medium	Tobitec FedVigor02	LQ47-125×2	2 ~ 8℃, away from light
Transfection enhancer	CHE STE01	ML701-20×1	2 ~ 8℃, away from light
Transfection reagent （1mg/mL）	Tobitec TR01	ML801-2×2	2 ~ 8℃, away from light

Product Introduction

The HEK293 Transient Transfection Kit (HEK Trans kit01) is chemically component-limited and is suitable for high-density culture and transient transfection expression of different subtypes of HEK293 cells. 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℃, away from light. Store away from light (for storage temperature, refer to [Packaging Specifications]). The validity period is 6 months.

Quality Index

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Product Index	HEK293 CDM26 Pro (LQ26-2), 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 HEK293 CDM26 Pro(LQ26-2) 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.

4. Pipet the contents from the vial gently into a centrifuge tube containing 30mL of pre-warmed medium HEK293 CDM26 Pro(LQ26-2).

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 HEK293 CDM26 Pro(LQ26-2), 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 HEK293 CDM26 Pro(LQ26-2) 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 HEK293 CDM26 Pro(LQ26-2) medium.

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

6. Passage cells with fresh HEK293 CDM26 Pro(LQ26-2) 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 HEK293 CDM26 Pro(LQ26-2) medium, passage cells after sampling and counting.

Adaptation

Direct Adaptation

1. For cells can direct adapt, transfer cells suspension cultures into HEK293 CDM26 Pro(LQ26-2) 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.

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 HEK293 CDM26 Pro(LQ26-2) 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 HEK293 CDM26 Pro(LQ26-2) 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%HEK293 CDM26 Pro(LQ26-2) + 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.

Refer to the transient transfection strategy

1. After HEK293 cells have fully adapted, they are subcultured and expanded at a density of 0.5×10^6 cells/mL. When the cell volume meets the requirement for transfection, the cell density is adjusted one day before transfection. Specifically, on the day before transfection, the cell density is diluted to 2×10^6 cells/mL using fresh HEK293 CDM26 Pro (LQ26-2) medium, ensuring that the viable cell density on the day of transfection remains

between 4 ~ 6×10⁶ cells/mL.

2. Perform the transfection operation (taking a 30mL culture volume as an example). The optimal transfection conditions should be optimized according to specific circumstances, and the following transfection conditions are for reference only:

VCD	DNA	Tobitec TR01
4 ~ 6 x10⁶ cells/mL, viability > 95%	1.3 mg/L	3.9 mg/L

2.1. On the day of transfection (D0), first count the cells to ensure the viable cell density is between 4 ~ 6×10⁶ cells/mL. Dilute 39 μg of plasmid DNA with 1.5 mL of HEK293 CDM26 Pro (LQ26-2) medium and mix well. Then, dilute 117 μL of the transfection reagent Tobitec TR01 (1 mg/mL) with 1.5 mL of HEK293 CDM26 Pro (LQ26-2) medium and mix well. Add the mixed Tobitec TR01 to the well-mixed DNA solution, mix gently and slowly until uniform, and let it stand for incubation for 10 ~ 15 minutes.

2.2. Take out the cell suspension prepared for transfection, slowly add the DNA and Tobitec TR01 complex dropwise into the cell culture medium, and shake the cell suspension while adding until the dropwise addition is complete. Place the shake flask under the specified environmental conditions (see "Cell Culture Conditions" in the table above) for cell culture. The shaker is set to a temperature of 36.5°C or 37°C, and there is no temperature reduction during the culture process.

3. Add transfection enhancer CHE STE01 at 2% of the culture system within 8 ~ 24 hours after transfection (only once) and feed according to Process 1.

Appendix: Reference Feeding Process:

Test Process	Reference Feeding Process
Process 1	Add 7% Tobitec FedVigor02 each time on D1, D4 and D7 (recommended).
Process 2	Add 3% Tobitec FedVigor02 each time on D1, D3, D5 and D7 (Alternative 1).
Process 3	Add 5% Tobitec FedVigor02 each time on D3, D6 and D9 (Alternative 2).

Note: When the cell viability is ≤70%, the culture can be terminated, and the expression level of the target product for testing should be determined.

【Technical Support】：

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

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