Version 2，2023

The User's Manual—HEK293 CDM26

Basic Information Introduction

Product Introduction

HEK293 CDM26 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 different subtypes HEK293 cell lines (HEK293F, HEK293T, HEK293H, Expi293 and ect.), it can effectively support high-level expression of recombinant proteins, lentivirus and adenovirus, it can also be used in the development and production process of adenovirus related products. HEK293 CDM26 combine with commercial feed media, can support high-density cell expansion, viability maintenance, and achieve efficient expression of target products.

Application Scope

HEK293 CDM26 can be used in the process of HEK293 cell line cell thawing, cell passage, high-density cell suspension culture expansion and transient expression. It can be used in the research and further manufacturing of recombinant proteins, lentivirus and adenovirus 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
HEK293 CDM26	LQ26, Liquid	2°C ~ 8°C, Protect from light	2°C ~ 8°C, Protect from light	12 months
HEK293 CDM26	DP26, Powder	2°C ~ 8°C, Protect from light	2°C ~ 8°C, Protect from light	24 months

Protocol for Hydration of Powder Medium

1. Fill the mixing container with purified water (20 ~ 30°C) at 90% of the final volume.
2. Slowly add 23.00 g/L of powder medium with gentle stirring. Mix for 10 minutes.
3. Adjust the pH to 7.0 ~ 7.3 using 5M NaOH solution. Mix for more than 10 minutes.

4. Slowly add 2.30 g/L $NaHCO_3$ to the solution with gentle stirring, mix for 10 minutes.

5. Adjust the pH to 7.1 ~ 7.4 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	HEK293 CDM26 (LQ26), Liquid	HEK293 CDM26 (DP26), Powder
Appearance	Light yellow, clear liquid	Off white to light yellow powder
pH	6.9 ~ 7.5	7.1 ~ 7.6 (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	< 10
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	36 ~ 38℃

	Incubation CO_2 concentration	5%
	Incubation relative humidity	> 80% RH

Cell Thawing

1. Pre-warm the medium (HEK293 CDM26) 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 (HEK293 CDM26).

5. Centrifuge 150 g ~ 300 g for 5 minutes. Discard the supernatant and re-suspend cells in 10 ~ 30mL fresh pre-warmed medium (HEK293 CDM26), 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) 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 HEK293 CDM26 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 HEK293 CDM26 medium, passage cells after sampling and counting.

Adaptation

Direct Adaptation

1. For cells can direct adapt, transfer cells suspension cultures into HEK293 CDM26 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 HEK293 CDM26 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 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 + 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 Transient Transfection and Fed Feeding Strategy

HEK293 Transient and Expression of Proteins

1. HEK293 cells that have been adaptively cultured can be transfected after 3 more passages.

2. Seed cells at 0.5×10^6 cells/mL into HEK293 CDM26.

3. After 2 ~ 3 days of cell culture, the viable cell density can reach $2 \sim 4\times10^6$ cells/mL, dilute the culture media with

the same volume of pre-warmed HEK293 CDM26 medium (volume ratio = 1 : 1), continue to culture the cells, and take daily samples to detect the viable cell density (×10^6 cells/mL) and cell viability (%).

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
4.5x10^6 cells/mL, cell viability > 95%	1.5 mg/L	4.5 mg/L

5. Fed Feeding Strategy

Method 1: Tobitec FD & Tobitec FB fed feeding strategy

Feeds/Time	D1	D5
Tobitec FD	5%	5%
Tobitec FB	0.5%	0.5%
Glutamine	4mmoL/L	4mmoL/L
Glucose	When the glucose concentration is below 3 g/L, glucose is added at the final concentration of 5 g/L	
Parameter recommendations: incubation temperature after transfection 36.5℃, pH: 7.1±0.2, DO: 40%.		

Note: If cell culture time is less than 5 days after transfection, Tobitec FB may not be added.

Method 2: Tobitec FC Pro fed feeding strategy

Feeds/Time	D0	D1	D3
Tobitec FC Pro	--	5 ~ 8%	5 ~ 8%
Glutamine	--	4mmoL/L	4mmoL/L
Glucose	When the glucose concentration is below 3 g/L, glucose is added at the final concentration of 5 g/L		
Parameter recommendations: incubation temperature after transfection 36.5℃, pH: 7.1±0.2, DO: 40%.			

Virus Infection and Expression

1. HEK293 cells that have been adapted to HEK293 CDM26, virus infection can be performed after 3 more passages.

2. Seed the fully adapted cells at 0.5×10^6 cells/mL into HEK293 CDM26.

3. After 2 ~ 3 days of cell culture, the viable cell density can reach 2 ~ 4×10^6 cells/mL, dilute the culture media with

the same volume of pre-warmed HEK293 CDM26 medium (volume ratio = 1 : 1), continue to culture the cells, and take daily samples to detect the viable cell density ($\times 10^6$ cells/mL) and cell viability (%).

4. Viral transient: explore the best transfection conditions according to the specific situation and perform transfection.

5. Feeding strategy:

Method 1: Tobitec FA & Tobitec FB fed feeding strategy

Feeds/Time	D1	D5
Tobitec FA	5%	5%
Tobitec FB	0.5%	0.5%
Glutamine	4mmoL/L	4mmoL/L
Glucose	When the glucose concentration is below 3 g/L, glucose is added at the final concentration of 5 g/L	
Parameter recommendations: incubation temperature after transfection 36.5℃, pH: 7.1±0.2, DO: 40%.		

Note: If cell culture time is less than 5 days after transfection, Tobitec FB may not be added.

Method 2: Tobitec FC fed feeding strategy

24h after transfection, add Tobitec FC feeding product at 5 ~ 8%, and add 4 mmoL/L glutamine according to the culture volume.