Optimization
Makes Differences

OPM-293 CD03

Chemically Defined Cell Culture Medium

—— For Biomanufacturing with HEK293 cell lines

OPM-293 CD03 is chemically defined, free of any animal-origin components, and contains no hydrolysates, proteins, growth factors or components of unknown composition. It can be used to reach & maintain high density suspension culture of HEK293 cell lines and to achieve highly efficient transient transfection.

Application

OPM-293 CD03 is intended for large scale manufacturing of therapeutic biomolecules, as well as for research purposes, but not for human or any therapeutic use.

Storage & Transportation

Store at 2~8℃, dark and dry
Ship at Room temperature (Liquid), Blue ice (Dry powder)

Shelf Life

OPM-293 CD03 Medium Liquid: 12 months
OPM-293 CD03 Dry Powder: 24 months

Reconstitution Method for Dry Powder

1. Take 90% of the final prepared volume of ultra-pure water or WFI, the temperature is 25~35°C (Note: Prepared volume is not less than 1 L).

2. Add 17.93 g/L dry powder medium slowly to water. Keep stirring.

3. Add 0.877 g/L L-glutamine slowly to the vessel. Keep stirring.

4. Add 2.22 g/L Sodium Bicarbonate to the vessel. Keep stirring for 10 minutes.

5. Add 5N NaOH slowly to adjust the pH until the solution is clear. Adjust pH to 7.0 with 5N HCl slowly.

6. Adjust to the final volume with ultra-pure water or WFI. Then adjust the osmolality to 285 ± 10 mOsm/kg with calculated amount of NaCl.

7. Mix for an additional 10 minutes. Sterilize immediately by membrane filtration.

Quality Specifications

Specifications	OPM-293 CD03 Medium	OPM-293 CD03 DPM
Appearance	Red clear liquid	Off -white or light yellow powder
pH	7.0~7.4	7.0~7.4
Osmolality (mOsm/kg)	270~300	270~300
Solubility	——	Good by following the reconstitution instructions
Endotoxin (EUcells/mL)	＜1.0	<1.0
Sterility test	Negative	——

Cell Culture Conditions

37℃, 80% humidity, 5~8%CO_2

Shaker speed 110–150 rpm (amplitude: 50mm).

Cell Recovery

1. Rapidly thaw (<2 minute) a vial of frozen cells in a 37 °C water bath.
2. Transfer the entire contents aseptically into a 125mL shake flask containing 30 mL prewarmed OPM-293 CD03 Basal Medium.
3. Incubate at 37 °C in a humidified atmosphere of 5~8% CO_2 in air on a shaker (rotating at 115~125 rpm, amplitude: 50mm).

Cell Culture Passaging

1. Cultures should be passaged during the mid-log phase, approximately every 2~3 days. Be aware that different HEK293 cell lines may have different logarithmic growth phases which require individual calculation.
2. Determine the viable cell density (VCD) and the cell viability.
3. Proceed if VCD= 3 x 10^6 ~ 4 x 10^6 cells/mL & viability ≥95%. Otherwise troubleshoot conditions before continuing.
4. Determine the correct volume of cell culture to inoculate a new flask at a starting cell density of 0.3 x10^6 ~ 0.6 x10^6 cells/mL in prewarmed OPM-293 CD03 Basal Medium.

Medium Adaptation

Direct Medium Adaptation

1. In most cases, HEK293 cell lines may be adapted directly from previous medium into OPM-293 CD03 Basal Medium.
2. Adaptation should begin when cells are in mid-log phase, and viability ≥ 95%.
3. Adaptation is completed when the cultures attain stable VCD of 3×10^6cells/mL and viability ≥ 95% within 3~4 days over at least 2~3 passages.

Sequential Medium Adaptation

1. For certain HEK293 cell lines failing the direct medium adaptation, sequential adaptation is recommended.
2. Passage the cells in current media for 2~3 passages to reach stable cell growth before beginning with medium adaptation.
3. The adaptation instruction below provided below relies on maintaining the cell culture in mid-log growth phase by passaging the cells every 3 to 4 days. At least two passages at each adaptation step are recommended.
4. Adaptation is completed when the cultures attain stable VCD of 3×10^6cells/mL and viability ≥ 95% within 3~4 days over at least 2~3passages.

Recommended Transient Transfection Protocol

OPM-293 CD03 : current media (%)	Seeding density (×10^6 cells/mL)	Evaluation of cell growth	Acceptance criteria for next step
0:100	as usual	VCD & Viability	VCD≥3×10^6/mL, Viability≥95% over 2 passages
30:70	0.6	VCD & Viability	VCD≥3×10^6/mL, Viability≥95% over 2 passages
70:30	0.5	VCD & Viability	VCD≥3×10^6/mL, Viability≥95% over 2 passages
100:0	0.4	VCD & Viability	VCD≥3×10^6/mL, Viability≥95% over 2 passages

Recommended transfection condition

The optimal transfection conditions should be optimized case by case and may need to be established by DOE method. The following transfection conditions are only for reference:

VCD	2 x10^6 ~ 4 x10^6 cells/mL
DNA	0.7 ~ 2 mg/L
DNA/PEI ratio	1/2 ~ 1/6

Recommended transfection and feeding strategy

Feed	Feeding strategy
OPM-293 ProFeed	24h/48h post-transfection add 5% OPM-293 ProFeed

Recommended transfection procedure

Time line	Steps	Instruction
Preparation	**1**	Culture HEK293 cells used for transfection until stable VCD of 3x10^6 ~ 4 ×10^6 cells/mL and viability ≥ 95%
Day -1	**2**	24 hours prior to transfection, split cells to a density of 3 x10^6 ~ 4x10^6 cells/mL and culture overnight in shake flasks.
Day 0	**3**	At the time of transfection, cell density should be 3 x10^6 ~ 4x10^6 cells/mL & with viability ≥ 95%. In case of higher VCD, dilute the cell suspension with fresh medium.
	4	Prepare the dilution of the expression vector plasmid DNA using OPM-293 CD03 Basal Medium. Mix carefully.
	5	Prepare a dilution of the PEI stock solution using OPM-293 CD03 Basal Medium. Mix carefully.
	6	Add diluted PEI to the diluted DNA plasmid solution, mix carefully. Incubate at room temperature 20 minutes.
	7	Add the DNA-PEI mixture to the cell suspension prepared in step 3 while swirling the culture to mix.
	8	Return the shake flask to 37°C incubator.
Day 1	**9**	16~24 hours post transfection, add 5% (v/v) OPM-293 ProFeed to the culture flask while slightly swirling. Return the flask to the 37°C incubator.
Day2-7	**10**	Maintain the glucose concentration above 4g/L. Harvest cells when the viability drops below 60%.

Order Information

Cell Culture Media

Name	Cat No.	Type	Volume	Description
OPM-293 CD03 DPM	91070-010	Dry powder	10L	Without L-Glutamine
	91070-050	Dry powder	50L	
OPM-293 CD03 Medium	81070-001	Liquid	1000mL	Without L-Glutamine

High Performance Feeds

Name	Cat No.	Type	Volume	Description
OPM-293 ProFeed	F081918	Liquid	100mL	Protein-free feed
	F081918-001	Liquid	1000mL	

Other HEK293 Culture Medium

Name	Cat No.	Type	Volume	Description
OPM-293 CD05 Medium	81075-001	Liquid	1000mL	With L-Glutamine
OPM-CD Trans293	P82019	Liquid	1000mL	With L-Glutamine

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