

OPM-CD Trans293

Chemically Defined Cell Culture Medium

— For Biomanufacturing with HEK293 cell lines



OPM-CD Trans293 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. This liquid medium contains L-Glutamine.

Application

OPM-CD Trans293 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°C, dark and dry
Ship at Room temperature (Liquid), Blue ice (Dry powder)

Shelf Life

OPM-CD Trans293 Medium Liquid: 12 months

Quality Specifications

| Specifications | OPM-CD Trans293 Medium |
|----------------------|------------------------|
| Appearance | Red clear liquid |
| pH | 7.0~7.4 |
| Osmolality (mOsm/kg) | 270~300 |
| Solubility | --- |
| Endotoxin (EU/mL) | <1.0 |
| Sterility test | Negative |

Cell Culture Conditions

37°C, 80% humidity, 5~8%CO₂
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-CD Trans293 Basal Medium.
3. Incubate at 37 °C in a humidified atmosphere of 5~8% CO₂ 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.

- Determine the viable cell density (VCD) and the cell viability.
- Proceed if VCD= $3 \times 10^6 \sim 4 \times 10^6$ cells/mL & viability $\geq 95\%$. Otherwise troubleshoot conditions before continuing.
- Determine the correct volume of cell culture to inoculate a new flask at a starting cell density of $0.3 \times 10^6 \sim 0.6 \times 10^6$ cells/mL in prewarmed OPM-CD Trans293 Basal Medium.

Medium Adaptation

Direct Medium Adaptation

- In most cases, HEK293 cell lines may be adapted directly from previous medium into OPM-CD Trans293 Basal Medium.
- Adaptation should begin when cells are in mid-log phase, and viability $\geq 95\%$.
- Adaptation is completed when the cultures attain stable VCD of 3×10^6 /mL and viability $\geq 95\%$ within 3~4 days over at least 2~3 passages.

Sequential Medium Adaptation

- For certain HEK293 cell lines failing the direct medium adaptation, sequential adaptation is recommended.
- Passage the cells in current media for 2~3 passages to reach stable cell growth before beginning with medium adaptation.
- 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.
- Adaptation is completed when the cultures attain stable VCD of 3×10^6 /mL and viability $\geq 95\%$ within 3~4 days over at least 2~3 passages.

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

Recommended Transient Transfection Protocol

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 | $2 \times 10^6 \sim 4 \times 10^6$ 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 $3 \times 10^6 \sim 4 \times 10^6$ cells/mL and viability $\geq 95\%$ |
| Day -1 | 2 | 24 hours prior to transfection, split cells to a density of $3 \times 10^6 \sim 4 \times 10^6$ cells/mL and culture overnight in shake flasks. |
| Day 0 | 3 | At the time of transfection, cell density should be $3 \times 10^6 \sim 4 \times 10^6$ cells/mL & with viability $\geq 95\%$. In case of higher VCD, dilute the cell suspension with fresh medium. |
| | 4 | Prepare the dilution of the expression vector plasmid DNA using OPM-CD Trans293 Basal Medium. Mix carefully. |
| | 5 | Prepare a dilution of the PEI stock solution using OPM-CD Trans293 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

OPM-CD 293 Transient Transfection System

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

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