Optimization
Makes Differences

DMEM

High-glucose Basal Cell Culture Medium

—— For Biomanufacturing

DMEM (Dulbecco's Modified Eagle Medium) is a high-glucose basal cell culture medium that contains 4.5 g/L glucose and L-glutamine, no sodium pyruvate. DMEM supports the growth of adherent cells. DMEM does not contain proteins, lipids or any growth factors, so it might need to be used with serum.

Application

DMEM 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

DMEM Medium Liquid: 12 months
DMEM Dry Powder: 24 months

Reconstitution Method for Dry Powder

1. Measure out 90% of final required volume of purified water intended for cell culture use, e.g. WFI. Recommended water temperature is 25~35℃ (minimum final volume ≧ 1L) .

2. Slowly add dry powder at 13.375 g/L while stirring, and continue mixing for 10 minutes. Residual powder attached to the vessel wall should be taken into the solution.

3. Add 3.7 g/L NaHCO3 to the solution, and continue to stir for 20~30 minutes until completely dissolved..

4. Adjust pH to 7.0 by slowly adding 5N HCl.

5. Add cell culture grade purified water to 100% final volume. Continue to stir for 10 minutes.

6. Sterile filter using a membrane filter with a pore size of 0.22 micron.

Quality Specifications

Specifications	DMEM (1X) Medium	DMEM DPM
Appearance	——	Off -white or light yellow powder
pH	7.0~7.5	7.0~7.5
Osmolality (mOsm/kg)	310~355	310~355
Solubility	——	Good by following the reconstitution instructions
Endotoxin (EU/mL)	＜1.0	＜1.0
Sterility test	Negative	——

Cell Culture Conditions

37℃，95% humidity，5%CO_2

Cell Recovery

1. Prewarm the DMEM medium at 37°C, 5% CO_2 for 30min.
2. Rapidly thaw frozen vial of frozen cells in a 37°C water bath.
3. Transfer the entire contents aseptically into a sterile centrifuge tube containing 5~10 mL prewarmed DMEM medium.
4. Centrifuge at 800 rpm for 5 minutes and discard the supernatant.
5. Resuspend the cell pellet with an appropriate amount of fresh medium, and transfer to a suitable culture vessel.
6. Add an appropriate amount of serum, shake the vessel gently to mix the cells, and incubate it in a humidified 37 °C incubator with 5% CO_2.
7. Passage the cells when adhere to the monolayer and reach 80% confluency.

Cell Culture Passaging

1. Prewarm the DMEM medium at 37°C, 5% CO_2 for 30min.
2. Aspirate medium from cell monolayer and rinse the culture vessel three times with prewarmed DPBS without Ca^{2+} or Mg^{2+}.
3. Add 0.25% Trypsin-EDTA to the culture vessel and incubate until cells have detached (~2–5 minutes at room temperature).
4. Tilt the culture vessel to make the cell supernatant flow out as soon as possible when the dissociation exceeds 90%. Stop the dissociation reaction by adding prewarmed DMEM medium.
5. Centrifuge at 800 rpm for 5 minutes and discard the supernatant.
6. Resuspend the cell pellet with an appropriate amount of fresh medium, and aliquot into new culture vessels.
7. Add an appropriate amount of fresh medium and serum, shake the vessels gently to mix the cells, and incubate them in a humidified 37 °C incubator with 5% CO_2.

Cryopreservation

1. Harvest the desired quantity of cells in log phase of growth with viability over 90%.
2. Determine VCD to ensure that the final cell density is (0.5~1.5)x10^7 cells/ml.
3. Prepare the freezing medium consisting of 80% DMEM medium, 10%FBS and 10% DMSO. Let the freezing medium cool down to 4℃.
4. Add 0.25% Trypsin-EDTA and incubate until cells have detached (~2–5 minutes at room temperature).
5. Resuspend the cells with an appropriate amount of fresh medium. Centrifuge at 800 rpm for 5 minutes and discard the supernatant.
6. Resuspend the cell pellet in the pre-determined volume of 4°C cryopreservation medium.
7. Dispense aliquots of this suspension into cryo-vials ((1~2 mL per vial).
8. Place the vials in a cryo-box or a controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
9. For long-term storage, transfer the vials to liquid nitrogen.

Order Information

Cell Culture Media

Name	Cat No.	Type	Volume
DMEM (1X)	P081702-001	Liquid	1000ml
DMEM	P150509	Dry powder	10L/50L/100L

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