

# MetaCell<sup>®</sup> DMEM

## Product Description

MetaCell<sup>®</sup> DMEM (Dulbecco's Modified Eagle Medium) is a well-defined, high-glucose basal medium containing 4.5 g/L glucose, 110 mg/L sodium pyruvate, L-glutamine, and phenol red. It supports the growth of fast-growing, low-adherence adherent cells. DMEM does not contain proteins, lipids, or any growth factors, so it should be used in conjunction with serum.

This product is intended for research or further manufacturing but not for human or therapeutic use.

Product Name	Cat No.	Form	Size	Storage	Shelf Life
MetaCell <sup>®</sup> DMEM	P8000-X100	Powder	100L	2-8°C, protected from light	12 months

## Cell Culture Conditions

37 ± 0.5°C, 5-10% CO<sub>2</sub>, humidity 95%.

## Media preparation instruction (1L of final volume of liquid medium)

1. Take 90% of the final preparation volume of ultra-pure water or water for injection, ensuring the water temperature is between 25-35°C. Note: the minimum single preparation volume should not be less than 1 L.
2. Weigh 13.485 g of dry powder medium per liter and slowly add it to the water while continuously stirring to ensure even dispersion.
3. Weigh 3.7 g of sodium bicarbonate per liter, add it to the solution, and continue stirring to dissolve completely.
4. Stir the mixture for 20 minutes, then adjust the pH to the desired level using 5 mol/L sodium hydroxide as needed.
5. Top up with ultra-pure water or water for injection to achieve the final preparation volume.
6. Continue stirring for an additional 10 minutes to ensure thorough mixing, then sterilize the solution by filtering through a 0.22 µm filter into a suitable container.

## Cell Recovery

1. Pre-warm the MetaCell<sup>®</sup> DMEM in a 37°C incubator with 5% CO<sub>2</sub> for 30 minutes.
2. Remove the cryovial containing the frozen cells and immediately transfer it to a 37°C water bath until completely thawed.
3. Transfer the thawed cell suspension into a sterile centrifuge tube containing 5-10 mL of fresh pre-warmed MetaCell<sup>®</sup> DMEM.
4. Centrifuge at 800 rpm for 5 minutes at room temperature, then carefully aspirate and discard the supernatant.

5. Resuspend the cell pellet in an appropriate volume of fresh MetaCell® DMEM supplemented with serum. Transfer the resuspended cells to a suitable culture vessel, gently swirl to mix, and incubate at 37°C with 5% CO<sub>2</sub>.
6. Monitor the cells under a microscope. When they form a monolayer and reach approximately 80% confluence, passage them as needed.

### **Cell Cryopreservation**

1. Prepare a sufficient number of cells in the early logarithmic growth phase with a cell viability >90% for cryopreservation.
2. The final cell concentration for cryopreservation should be controlled at  $5.0\text{--}15.0 \times 10^6$  cells/mL.
3. Cryopreservation solution: (80% MetaCell® DMEM + 10% DMSO + 10% FBS), precool at 4°C.
4. Harvest adherent cells that are growing well by treating them with 0.25% trypsin-EDTA until they detach. Resuspend the cells in fresh culture medium, then centrifuge the suspension at 800 rpm for 5 minutes. After centrifugation, carefully aspirate and discard the supernatant.
5. Resuspend the cell pellet in an appropriate volume of cell cryopreservation solution. Take a sample for counting, and adjust the cell density to the target value.
6. Quickly aliquot the cell suspension into cryovials, with 1-2 mL per vial.
7. Place the cryovials in a programmed cooling box and store them overnight at -80°C. Afterward, transfer the cryovials to a liquid nitrogen tank for long-term storage.

### **Cell Culture**

1. Under a microscope, select adherent cells that are growing well, forming a monolayer with approximately 80% confluence. Choose an appropriate culture vessel based on your needs.
2. Place the MetaCell® DMEM in a 37°C incubator with 5% CO<sub>2</sub> for 30 minutes to pre-warm it.
3. Aspirate and discard the original medium from the culture vessel.
4. Rinse the cells three times with calcium- and magnesium-free balanced salt solution (PBS).
5. Add 0.25% trypsin-EDTA to the culture vessel and incubate at room temperature for 2 minutes (the actual incubation time may vary depending on the cell line).
6. Observe the cells under a microscope. When more than 90% of the cells have detached, tilt the culture vessel to allow the liquid to drain quickly. Immediately add an appropriate volume of pre-warmed MetaCell® DMEM and gently pipette the surface to disperse the cells.
7. Centrifuge the cell suspension at 800 rpm for 5 minutes at room temperature. Carefully aspirate and discard the supernatant. Resuspend the cell pellet in pre-warmed MetaCell® DMEM and aliquot into new culture vessels, adding fresh medium and serum as needed.
8. Gently swirl the container to mix the cells evenly, then transfer it to a 37°C incubator with 5% CO<sub>2</sub> for continued culture.