



**Mi-T-MEFs Cells  
(Cat# A-6)**

**Instructions for Use:**

**Contents**

- 1 vial of Mi-T-MEFs Cells(7.2x10<sup>6</sup> cells/vial)

**Required but not Supplied**

- DMEM/High Glucose medium
- ES qualified FBS
- L-Glutamine (200mM)

**Optional but not Supplied**

- Penicillin (5,000 U/ml)/Streptomycin (5,000 µg/ml)

**Unpacking and Storage Instructions**

**Mi-T-MEFs Cells**

- Cells must be moved from dry ice to liquid nitrogen IMMEDIATELY. Temperature fluctuations will have adverse effects on cell health and viability.
- When stored in the recommended storage conditions (liquid nitrogen), Mi-T-MEFs Cells can remain stable in excess of 3 years.

**Supplementing DMEM/High Glucose Medium**

1. Decontaminate the external surfaces of all supplement vials and the medium bottle with ethanol or isopropanol.
2. Aseptically open each supplement vial and add the amount indicated below to the basal medium with a pipette.

<b>To make 100 mL of complete medium:</b>	
DMEM/High Glucose medium (Hyclone Cat# SH30081.01)	90 mL
ES Qualified FBS (Hyclone Cat# SH30070.03E)	10 mL
L-Glutamine	2 mL
Penicillin/Streptomycin (optional)	1 mL

3. Supplemented medium should be stored at 2-8°C, protected from light. The complete medium should be given a 2 week expiration date. Dispense the complete medium into aliquots to avoid repeated heating prior to each use.

## **Cell Thawing Protocol**

### **Protocol Description:**

Mi-T-MEFs Cells form adherent monolayer cultures when grown on cell culture plates. We recommend thawing your Mi-T-MEFs Cells using the following protocol.

<b>Required but not supplied:</b>
Cell culture treated plates

### **To Plate the cells perform the following steps:**

1. Do not thaw the cells until the recommended medium and appropriately coated plasticware and/or glassware are on hand.
2. Remove the vial from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with ethanol or isopropanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful to not introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of fully supplemented DMEM/High Glucose (pre-warmed to 37°C) to the 15 mL conical tube.

*IMPORTANT: Do not add the whole volume of medium at once to the cells. This may result in decreased cell viability due to osmotic shock.*

6. Gently mix the cell suspension by slow pipetting up and down twice. Be careful to not introduce any bubbles.

*IMPORTANT: Do not vortex the cells. Breaking cells down to single cell suspensions will significantly increase cell death.*

7. Centrifuge the tube at room temperature at 200 x g for 4 minutes to pellet the cells.
8. Aspirate as much of the supernatant as possible. Steps 4-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in a total volume of 2 mLs of fully supplemented DMEM/High Glucose (pre-warmed to 37°C).
10. We recommend plating a vial of Mi-T-MEFs Cells onto a 100 mm dish with a total of 10 mL of media.
11. Incubate the cells at 37°C in a 5% CO<sub>2</sub> humidified incubator.
12. Exchange the medium with fresh fully supplemented DMEM/High Glucose medium (pre-warmed to 37°C) 24 hours post plating. Exchange with fresh medium every other day thereafter. Use caution not to dislodge the cells; do not pipette media directly onto the cells but rather onto the side of the culture dish.
13. Seed pluripotent cells on Mi-T-MEFs Cells 2-3 days post thaw.

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