Miltenyi Biotec

MACS® Freezing Solution

Order no. 130-129-552

Contents

1. Description

This product is for research use only.

Components	**50 mL MACS Freezing Solution**
Specifications	pH: 7.4–7.8 Contains 10% DMSO as cryoprotectant.
Storage	Store protected from light at 2–8 °C. The expiration date is indicated on the vial label.

1.1 Background information

The MACS Freezing Solution is a ready-to-use animal component-free media formulation designed for the xeno- and serum-free cryopreservation of primary cells and solid tissues, such as human and mouse tumor samples, peripheral blood mononuclear cells (PBMCs), and cells from dissociated solid tissues (e.g. cells from dissociated tumors). Cells and tissues cryopreserved in MACS Freezing Solution show high preservation of viability and good recovery after thawing.

1.2 Applications

- Cryopreservation of primary cells for downstream applications (e.g. cell analysis, cell culture)
- Cyropreservation of solid tissues for downstream applications (e.g. dissociation into single-cell suspensions)

1.3 Reagent requirements

- Cell culture medium
- Cryogenic vials
- Freezing container
- (Only for tissue pieces) MACS SmartStrainers (70 µm) (#130-098-462)
- (Optional) MACS Tissue Dissociation Kits (e.g. Tumor Dissociation Kit, human (# 130-095-929)) in combination with the gentleMACS™ Octo Dissociator with Heaters (#130-096-427) for the preparation of single-cell suspensions from solid tissues

2. Protocol

▲ For the handling of the freezing container refer to the instructions of the provider.

2.1 Freezing of primary cells

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in MACS Freezing Solution to reach a concentration of 10^7 cells per mL.
4. Quickly transfer the cell suspension into cryogenic vials.
5. Place the vials in a freezing container and immediately store at –80 °C.
6. After 24 hours transfer the cryogenic vials with the cells into a liquid nitrogen tank for long-term storage.

2.2 Thawing of primary cells

▲ Work quickly to avoid loss of cells.

▲ Pre-heat water bath at 37 °C before taking cells out of liquid nitrogen.

1. Take a vial with cells out of the liquid nitrogen tank.
2. Incubate the vial in a pre-heated water bath at 37 °C until only a little lump of ice is left.
3. Quickly transfer cell suspension into a 15 mL conical tube and dropwise add 5 mL of cell culture medium.
4. Wash cryogenic vial with 1 mL of cell culture medium and add to conical tube.
5. Centrifuge cells for 5 minutes at 300×g. Aspirate supernatant carefully.
6. Resuspend the cell pellet in appropriate buffer according to downstream application.

2.3 Freezing of tissues

1. Determine tissue weight.
2. Cut tissue into small pieces as recommended for downstream application (e.g. tissue dissociation).

   ▲ **Note:** If using the MACS Tissue Dissociation Kits in combination with the gentleMACS Octo Dissociator with Heaters for downstream tissue dissociation, cut the tissue as indicated in the respective data sheet.
3. Transfer up to 200 mg of tissue pieces per 1 mL of capacity into the cryogenic vials.
4. Add 1 mL of MACS Freezing Solution to up to 200 mg of tissue pieces. For larger tissue amounts scale up accordingly.
5. Close the cryogenic vials and resuspend tissue pieces by inverting the vial several times until pieces float in MACS Freezing Solution.

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6. Place the vials in a freezing container and immediately store at –80 °C.
7. After 24 hours transfer the cryogenic vials with the tissue pieces into a liquid nitrogen tank for long-term storage.

2.4 Thawing of tissues

▲ Work quickly to avoid loss of cells.

▲ Pre-heat water bath at 37 °C before taking tissues out of liquid nitrogen.

1. Place a MACS SmartStrainer (70 µm) on a 50 mL conical tube.
2. Rinse the MACS SmartStrainer (70 µm) with 1 mL of cell culture medium.
3. Take a vial with tissue pieces out of the liquid nitrogen tank.
4. Incubate the vial in a pre-heated water bath at 37 °C until only a little lump of ice is left.
5. Pour tissue pieces onto the MACS SmartStrainer (70 µm).
6. Wash cryogenic vial with 1 mL of cell culture medium and pour it onto the MACS SmartStrainer (70 µm).
7. Wash tissue pieces with additional 15 mL of cell culture medium.
8. Pieces are ready to be used for downstream application, e.g., tissue dissociation using the MACS Tissue Dissociation Kits in combination with the gentleMACS Octo Dissociator with Heaters.
9. (Optional) To recover cells remaining in the medium, centrifuge the medium for 5 minutes at 300×g.
10. (Optional) Aspirate supernatant and resuspend cell pellet in appropriate buffer according to downsteam application.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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