Version Date: 02/27/25

**Product Information Sheet**

# Human Cell Cryopreservation Media

## Catalog Number: MR1014

| Product Overview | |
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| **Product Name** | **Human Cell Cryopreservation Media** |
| Catalog #s | MR1014 |
| Quantity | 100 mL |
| Product Form | Liquid |
| Cell Type | All primary human cells and induced pluripotent stem cells |
| Reagents Needed | Penicillin/Streptomycin/Amphotericin B solution or Penicillin/Streptomycin solution, 100x (not included)[1] |

## Product Description

**Cryopreservation Media**

Cryopreservation Media is a highly versatile, high-performance formulation designed to support the long-term storage of both adherent and suspension cells. This specialized media ensures maximum post-thaw viability and recovery across a wide range of cell types, including hematopoietic stem cells, mesenchymal stem cells, amniotic epithelial stem cells, cancer cells, endothelial cells, fibroblasts, and immune cells. It contains 10% DMSO, a cryoprotectant that helps prevent ice crystal formation, minimizing cellular damage during the freezing and thawing process.

Engineered for broad compatibility, our Cryopreservation Media is optimized for use with all CET.bio adherent and non-adherent cell lines, which are available separately. When used according to protocol, this media enables indefinite cryogenic storage at temperatures ranging from -80°C to -196°C. While cells can be kept at -80°C for short-term storage, optimal long-term preservation requires storage in the vapor phase of liquid nitrogen or in an ultra-low freezer capable of reaching temperatures below -150°C.

**Usage & Handling of Cryopreservation Media:**

To ensure the best results, Cryopreservation Media should be stored at -20°C and thawed properly before use. Extended exposure to room temperature should be avoided, as temperature fluctuations may impact performance. Before adding to any cell culture, the media should be equilibrated in a water bath set at 37°C. Following proper cryopreservation protocols will help maintain cell integrity and viability upon thawing.

**Recommended Uses:**

- Compatible with a broad range of cell types, including stem cells, immune cells, fibroblasts, and more [i] [ii] [iii].
- Designed for use with CET.bio's complete catalog of adherent and suspension cell lines.

**Shipping & Storage:**

- Shipped with dry ice to maintain stability during transport.
- Store at -20°C for optimal performance and longevity.

Note: Although investigators are welcome to use this product with other human primary cells, CET cannot guarantee this product's performance with an unknown cell type. Additionally, using third-party cell lines with this product will void CET's warranty should they not function as indicated. Please refer to CET's Terms & Conditions, available at www.cet.bio.

## Product Image


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**CELLULAR ENGINEERING TECHNOLOGIES, INC.**
2500 Crosspark Road, Suite E110 Coralville, IA 52241
T: (319) 665-3000 F: (319) 665-3003
Email: support@cet.bio

| Cryopreservation Media Preparation | |
|---|---|
| Media Preparation | • Hematocytometer or automated cell counter (recommended).<br>• Cryopreservation media should be stored in working aliquots at -20°C and not frozen and thawed repeatedly. To make working aliquots, thaw the entire 100mL bottle in a 37°C water bath.<br>• When no ice crystals are visible, dispense the cryopreservation media in 5mL working volumes using 15mL sterile conical tubes. This should be done in a laminar flow hood using a sterile technique. When ready to freeze cells, remove the volume of cryopreservation media you need and thaw in a 37°C water bath. |

| Freezing Non-Adherent Cells | |
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| 1. | Whether you plan on freezing adherent or non-adherent cells, using cells actively growing in the logarithmic growth phase is strongly recommended. |
| 2. | To freeze non-adherent cells, such as CD34 or CD133 cells, in a sterile manner, remove an aliquot of the cell culture and count using a hematocytometer or automated cell counter (not included). |
| 3. | Once you have a cell count, centrifuge the rest of the culture at 200 x g for 10 minutes using a swing bucket rotor. A swing bucket rotor is recommended but not required. |
| 4. | Carefully withdraw the media supernatant either with an aspirator or careful decanting. Pellets of non-adherent cells tend to be very loosely held together, so be careful not to lose your cell pellet. |
| 5. | Resuspend the cell pellet in pre-warmed Cryopreservation Media at the density you desire based on your cell count. We recommend a density of 100,000 to 1,000,000 cells per milliliter of Cryopreservation Media. |
| 6. | In a sterile manner, transfer 1 milliliter of resuspended cells into a labeled cryovial. CET recommends using high-grade cryovials that have a sealing gasket between the lid and body. |
| 7. | Transfer the tightly capped cryovial into a -80°C freezer for 24 hours. After that, frozen cryovials can be transferred into a liquid nitrogen storage unit or a freezer capable of temperatures lower than -150°C. Resuspended cells in cryovials must never be directly introduced to liquid nitrogen without curing in a -80°C freezer. |

| Freezing Adherent Cells | |
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| 1. | Wash the cell monolayer with Dulbecco's Phosphate-Buffered Saline (DPBS) (not included). Use a 10mL/T-75 flask. Rock the flask gently, then remove the DPBS and discard it. |
| 2. | Add 0.25% Trypsin/EDTA solution to a 5mL/T-75 flask. Rock the flask to spread the trypsin across the entire monolayer. Incubate at 37°C until the cells begin to detach. This should take approximately 5 minutes but no more than 15 minutes. Care must be taken that the cells are not forced to detach prematurely, which may result in clumping. |
| 3. | Inactivate the trypsin by adding at least an equal volume of complete growth media specific to that cell type. Pipette the cells up and down to separate them further into a single-cell suspension. |
| 4. | Remove an aliquot of the cell culture and count either using a hematocytometer or an automated cell counter. |
| 5. | Once you have a cell count, centrifuge the rest of the culture at 200 x g for 10 minutes using a swing bucket rotor. A swing bucket rotor is recommended but not required. |
| 6. | Carefully withdraw the media supernatant either with an aspirator or careful decanting. |
| 7. | Resuspend the cell pellet in pre-warmed Cryopreservation Media at the density you desire based on your cell count. We recommend a density of 100,000 to 1,000,000 cells per milliliter of Cryopreservation Media. |
| 8. | Transfer 1 milliliter of resuspended cells into a labeled cryovial in a sterile manner. CET recommends using high-grade cryovials with a sealing gasket between the lid and body. |
| 9. | Transfer the tightly capped cryovial into a -80°C freezer for 24 hours. After 24 hours, frozen cryovials can be transferred into a liquid nitrogen storage unit or a freezer capable of temperatures lower than -150°C. Resuspended cells in cryovials must never be directly introduced to liquid nitrogen without curing in a -80°C freezer. |

| Storage and Stability | | |
|---|---|---|
| | **Storage Temperature** | **Storage Time** |
| **Human Cell Cryopreservation Media** | -20°C | 6 months |



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*Avoid repeated exposure to room temperature and light.*

[1] These solutions should be portioned in 5mL aliquots, stored at -20°C, and never frozen/thawed. Although antimycotics are unnecessary, CET highly recommends their usage for long-term cell culture. Antibiotics and antimycotics should not be used as an alternative to proper aseptic techniques.



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