# Cryopreservation Media
## CRYO.Media-100

## Media Usage Protocol:

Cryopreservation media is designed to be used with all of CET's adherent and non adherent cells, all of which are available separately. When used as directed, this media will allow indefinite cryogenic storage of cells at temperatures below -80°C and up to -196°C. Although cells can be stored at -80°C for short periods of time, for optimal storage, cells should be stored in the vapor phase of liquid nitrogen or a freezer unit capable of achieving temperatures at low as -150°C at a minimum. The following is the recommended protocol for the usage of this media.

*Note: Cryopreservation media should be stored at -20°C. Avoid extended exposure of the media to room or higher temperatures. Media should be equilibrated in a water bath set at 37°C before adding to any cell culture.*

Figure 1: Cryopreservation Media



*Note: Antibiotics/ antimycotics should not be used as an alternative to proper aseptic technique.*

**Additional Equipment/Reagents Needed**

1. Hematocytometer or automated cell counter.
2. Cryopreservation media should be stored in working aliquots at -20°C and should not be freeze/thawed. To make working aliquots, thaw the entire 100mL bottle in a 37°C water bath. When there are no ice crystals visible, dispense the cryopreservation media in 5mL working volumes using 15mL sterile conical tubes. This should be done in a laminar flow hood using sterile technique. When you are ready to freeze cells, only remove the volume of cryopreservation media that you need and thaw in a 37°C water bath.

**Freezing Non-Adherent Cells**

1. Whether you plan on freezing adherent or non-adherent cells, it is best to use cells that are actively growing and in log phase.
2. For freezing non-adherent cells, such as CD34 or CD133 cells, in a sterile manner, remove an aliquot of the cell culture and count either using a hematocytometer or automated cell counter.
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 a 24 hour time period. After the 24 hour time period, 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.

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**Freezing Adherent Cells**

1. Wash the cell monolayer with Dulbecco's Phosphate Buffered Saline (DPBS). Use 10mL/T-75 flask. Rock the flask gently, then remove the DPBS and discard.
2. Add 0.25% Trypsin/EDTA solution at 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, as this 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 further separate into a single cell suspension.
4. Remove an aliquot of the cell culture and count either using a hematocytometer or 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. 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.
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**Certificate of Analysis**

All hematopoietic, mesenchymal and multipotent stem cells are evaluated by flow cytometry for specific stem cell markers. All other cells are evaluated either by staining, method of isolation or traditional molecular biology techniques. Data is available upon request.

All growth and differentiation media are evaluated by conducting assays to make sure cells either grow or differentiate as indicated on the media label. Data is available upon request.

All cells are tested for HIV-1, HIV-2, Hepatitis B and Hepatitis C using sensitive PCR based assays. All cells test negative for these viruses. However, all human cells must be used in accordance with established laboratory safety procedures and only under the supervision of trained personnel.





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