

# Human Mesenchymal Stem Cell Protocol: Cryopreservation

*Adapted from Kamath, A., Cellular Engineering Technologies, Inc.*

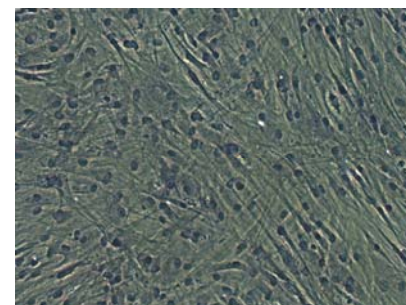
Thermo Scientific HyClone Cryopreservation Medium has been developed to support the cryopreservation of a variety of adherent cell types, including human Mesenchymal Stem Cells (hMSCs), Multipotent Cord Blood Unrestricted Somatic Stem Cells (MCBUSCs) and human Amniotic Epithelial Stem Cells.

*Note: Cryopreservation media should be stored at -20°C. Media must be thawed completely and equilibrated in a hot water bath at 37°C before use. Any remaining media should be aliquoted and stored at -20°C. Avoid freeze/thawing. All procedures must be done aseptically.*

## Subculturing

1. In a laminar flow hood, pipette spent medium from cell monolayer. Discard the spent medium.
2. Wash the monolayer with Thermo Scientific HyClone ES-Qualified DPBS (SH30850.03) by adding 10mL/75cm<sup>2</sup> to the flask, being careful not to disturb the monolayer. Rock the flask back and forth. Remove the DPBS from the monolayer and discard.

3. Add Thermo Scientific HyClone Trypsin (SH30042.01) at 3-5 mL/75 cm<sup>2</sup> flask and rock the flask to ensure that the entire monolayer is covered with the trypsin solution.
4. Incubate at 37°C until the cells begin to detach (approximately 5 minutes). Do not exceed 15 minutes. Care should be taken that the cells are not forced to detach prematurely, as this may result in clumping.
5. Add **complete** Thermo Scientific HyClone AdvanceSTEM™ Mesenchymal Stem Cell Expansion Media (Table 1) in equal the amounts to trypsin and pipette the cells up and down until the cells are dispersed into a single cell suspension.
6. To remove the trypsin, centrifuge cells at approximately 200 x g for 10 minutes at room temperature. Aseptically remove supernatant.
7. Resuspend the cell pellet in pre-warmed complete Thermo Scientific HyClone AdvanceSTEM™ Mesenchymal Stem Cell Expansion Media (Table 1) at approximately 5 mL/pellet for a 75 cm<sup>2</sup> flask. Remove a small volume sample for



**Figure 1:** hMSCs cultured in Thermo Scientific HyClone AdvanceSTEM™ Mesenchymal Stem Cell Basal Medium supplemented with 10 percent Thermo Scientific HyClone AdvanceSTEM™ Stem Cell Growth Supplement.  
*Photo courtesy of Cellular Engineering Technologies, Inc.*

counting.

8. Count the cells with a hemacytometer or cell counter and calculate cell count. Determine the density of cells required for cryopreservation. This will determine the volume of Thermo Scientific HyClone Cryopreservation Medium (SH30894.01) needed. A recommended density is on the order of 100,000 cells per 1 ml volume of cryopreservation medium.
9. Centrifuge the resuspended cells at 200 x g for 10 min.
10. Aspirate the medium leaving behind the cell pellet.

Resuspend the cell pellet using gentle pipetting in the appropriate volume of cryopreservation medium. Re-suspension is complete when no cell clumps are visible.

**Table 1: Preparation of 500 ml complete hMSC Expansion Medium**

Thermo Scientific HyClone AdvanceSTEM™ Mesenchymal Stem Cell Expansion Kit (SH30875.KT)			
Brand	Amount for 500 mL	HyClone Product	Catalog #
Thermo Scientific	450 mL	AdvanceSTEM™ Mesenchymal Stem Cell Basal Medium	SH30879.02 (1000 mL)
Thermo Scientific	50 mL	AdvanceSTEM™ Stem Cell Growth Supplement	SH30878.01 (100 mL)

**Store at 2-8°C. Discard unused medium after 8 weeks.**

11. Transfer suspended cells into sterile cryovials. A transfer volume of 1 ml in a 2 ml cryovial is recommended.

12. Once the cell suspension has been transferred to a sterile cryovial, transfer the cryovial to a -80°C freezer. Cryovials should be stored vertically for a 24 hour time period. At the end of 24 hours, transfer the tubes into the vapor phase of a liquid nitrogen storage unit for long term cryostorage.

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