

# Human Mesenchymal Stem Cell Protocol: Cryopreservation

Protocol  
SC 00007

*For research use only*

## *Background*

Thermo Scientific HyClone AdvanceSTEM Cryopreservation Medium (SH3089401) has been developed to support the cryopreservation of a variety of cell types, including human Mesenchymal Stem Cells (hMSCs), Multipotent Cord Blood Unrestricted Somatic Stem Cells (MCBUSCs) and human Amniotic Epithelial Stem Cells.

## *Required Materials*

- AdvanceSTEM™ Cryopreservation Medium (SH3089401)
- Thermo Scientific HyClone ES-Qualified DPBS (SH30850.03)
- Thermo Scientific HyClone Trypsin (SH30042.01)
- Thermo Scientific Nalgene “Mr. Frosty” Freezing Container (Fisher catalog no. 15-350-50)
- Cryopreservation vials (Fisher catalog no. 12-565-163N or equivalent)
- Thermo Scientific AdvanceSTEM Complete Mesenchymal Stem Cell Media

## *Media Preparation*

500 mL Complete Thermo Scientific HyClone AdvanceSTEM hMSC Expansion Medium

Thermo Scientific HyClone Product	Volume (500 mL final)	Catalog Number
AdvanceSTEM Mesenchymal Stem Cell Basil Medium	450 mL	SH30879.02 (1000 mL)
AdvanceSTEM Cell Growth Supplement	50 mL (10%)*	SH30878.01
Store at 2-8°C. Discard unused medium after 8 weeks.		

## *General Considerations*

AdvanceSTEM Cryopreservation Medium should be stored at -20°C. Media must be thawed completely and thoroughly resuspended. Any remaining media should be aliquoted and stored at -20°C. Avoid freeze/thawing.

Work with chilled vials, Mr.Frosty freezing containers and sterile tips.

Once the cells are in the cryopreservation media work gently.

## *Thawing Protocol*

1. In a laminar flow hood, pipette spent medium from the cell monolayer and discard.
2. Wash the monolayer with HyClone ES-Qualified DPBS. Use 10 mL/ T-75cm<sup>2</sup>. Rock the flask gently then remove the DPBS and discard.
3. Add Trypsin at 5 mL/ T-75cm<sup>2</sup>. 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 should be taken that the cells are not forced to detach prematurely, as this may result on clumping.
4. Inactivate the Trypsin by adding at least an equal volume of complete expansion media (Table 1). Pipette the cells up and down to further separate into a single cell suspension.
5. Resuspend the cells in a conical and centrifuge at 200 x g for 10 minutes. Remove supernatant.
6. Resuspend the cells in complete expansion media. Remove a small sample volume for counting (Table 1).

7. Count the cells with a hemacytometer or cell counter and calculate desired density to cryopreserve cells. A recommended density is in the order of  $5 \times 10^5$  cells/ 1 mL of cryopreservation media.
8. Centrifuge the resuspended cells at 200 x g for 10 minutes. Remove the supernatant and discard.
9. Resuspend the pellet gently in the appropriate volume of cryopreservation media and disperse into the cryopreservation vials.
10. Add the vials to the freezing container and inc at -80 °C for 24 hours.
11. Remove vials from -80 °C and store in liquid nitrogen.

*Related Protocols*

- SC Protocol 00009 - Human Mesenchymal Stem Cell Protocol: Sub Culturing hMSCs
- SC Protocol 00008 - Human Mesenchymal Stem Cell Protocol: Thawing and Plating

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