

Human Mesenchymal Stem Cells and Multipotent Cord Blood Unrestricted Somatic Stem Cell Protocol: Thawing and Plating

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Background

Human mesenchymal stem cells (hMSC) and multipotent cord blood unrestricted somatic stem cells (MCBUSSC) are primary cells which can be successfully cultured for approximately eight passages. The following is the recommended protocol for thawing and subculturing of these cells.

Required Materials

- Vial of human mesenchymal stem cells (hMSC) or multipotent cord blood unrestricted somatic stem cells (see Table 2)
- Complete hMSC expansion medium (see Table 1)
- General cell culture supplies

Media Preparation

Table 1: Complete hMSC Expansion Medium

Thermo Scientific HyClone AdvanceSTEM Mesenchymal Stem Cell Expansion Kit (SH30875.KT)		
Product Description	Volume (500 mL final)	Catalog Number
AdvanceSTEM™ Mesenchymal Stem Cell Basal Medium	450 mL	SH30879.02 (1000 mL)
AdvanceSTEM Stem Cell Growth Supplement	50 mL	SH30878.01 (100 mL)

Table 2: Available human mesenchymal stem cells

Cell Description	Size	Volume	Catalog Number
CET Human Wharton's Jelly Mesenchymal Stem Cells	2.0 mL vial	≥ 100,000 cells per mL	SV30101.01
		≥ 500,000 cells per mL	SV30101.02
CET Human Adipose-Derived Mesenchymal Stem Cells	2.0 mL vial	≥ 100,000 cells per mL	SV30102.01
		≥ 500,000 cells per mL	SV30102.02
CET Human Amniotic Mesenchymal Stem Cells	2.0 mL vial	≥ 100,000 cells per mL	SV30103.01
		≥ 500,000 cells per mL	SV30103.02
CET Human Bone Marrow Mesenchymal Stem Cells	2.0 mL vial	≥ 100,000 cells per mL	SV30110.01
		≥ 500,000 cells per mL	SV30110.02
CET Human Amniotic Epithelial Stem Cells	2.0 mL vial	≥ 100,000 cells per mL	SV30104.01
		≥ 500,000 cells per mL	SV30104.02
CET Multipotent Cord Blood Unrestricted Somatic Stem Cells	2.0 mL vial	≥ 100,000 cells per mL	SV30105.01
		≥ 500,000 cells per mL	SV30105.02
CET Human Cord Blood CD34+ Stem Cells	2.0 mL vial	≥ 100,000 cells per mL	SV30106.01
		≥ 500,000 cells per mL	SV30106.02
		≥ 1,000,000 cells per mL	SV30106.03
CET Human Cord Blood CD133+ Stem Cells	2.0 mL vial	≥ 100,000 cells per mL	SV30107.01
		≥ 500,000 cells per mL	SV30107.02
		≥ 1,000,000 cells per mL	SV30107.03

Special Considerations

- **Human bone marrow MSCs** can be sensitive coming out of cryogenic storage. Due to this, they tend to grow slowly over the first passage after thawing. To help alleviate this, we recommend these cells be thawed at a higher seeding density (at least 20,000 cells per cm²), pre-coating the plates and using a higher percentage of the growth supplement.
 - For example: 500,000 cells into a T-25 (20,000 cells per cm²) or 100,000 into a smaller area such as one well of a 12 well plate (33,000 cells per cm²).
 - To pre-coat the surface, we recommend pre-coating the cell culture surface with either gelatin or FBS 1 hour prior to seeding the flask, removing the gelatin or FBS and adding cells and media.
 - The concentration of the growth supplement can be increased to 15-20%. The medium should be exchanged every other day until cells are >90% confluent.

General Considerations

- Once complete media has been formulated, it should be stored at 2-8°C. Avoid extended exposure of the medium to room or higher temperatures. Medium should be equilibrated in a water bath set at 37°C before adding media to any cell culture.
- Antibiotics / antimycotics should not be used as an alternative to proper aseptic technique. However, should you prefer to add an antibiotic to your formulation, a concentration of 10 mL per liter is appropriate. Use Thermo Scientific HyClone Pen/Strep/Fungizone, SV30079.01
- Discard unused medium after 8 weeks.

Thawing Cells

1. Remove the vial of cells from dry ice. Defrost the vial of cells in a 37°C water bath with constant, moderate agitation, until ice in the ampoule is no longer visible.
2. Continue to warm the ampoule in the water bath for 30 seconds with gentle agitation.
3. Immediately disinfect the vial with 70% isopropanol.
4. Working in a laminar flow hood open the vial and transfer the contents to a sterile 15 mL tube.
5. Very slowly, add approximately 10 mL of complete MSC expansion medium (Table 1), pre-warmed to 37°C. Wait approximately 5 minutes for cells to acclimate.
6. Centrifuge the suspended cells at 200 x g for 10 minutes.
7. Decant the medium and gently re-suspend the cell pellet in 8-10 mL of complete MSC expansion medium (Table 1), then transfer into a 25 cm² culture flask (if working with bone marrow derived cells see special considerations above).
8. Observe the cells microscopically to estimate cell viability and then place the flask in an incubator at 37°C, 5% CO₂ and 90% humidity.
9. Cells will be ready to pass between 3-7 days. Cells should be subcultured at a density of 5,000 to 10,000 cells/cm² or desired plating density.

Related Protocols

- SC Protocol 00007 - Human Mesenchymal Stem Cell Protocol: Cryopreservation
- SC Protocol 00009 - Human Mesenchymal Stem Cell Protocol: Subculturing hMSCs

References:

Kamath, A., Cellular Engineering Technologies, Inc., <http://celleng-tech.com/index/index.html>