

# Human Mesenchymal Stem Cell Protocol: Subculturing hMSC's

Protocol  
SC 00009

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

For research use only

## Background

Human Mesenchymal Stem Cells (hMSC's) are primary cells which can be successfully cultured approximately eight passages. The following has been supplied to provide a protocol for the subculturing of these cells.

## Required Materials

- Human mesenchymal stem cells (hMSC's) (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

### General Considerations

- Any unused Thermo Scientific HyClone AdvanceSTEM Stem Cell Growth Supplement should be aliquoted and refrozen.
- Store all media at 2-8°C and avoid extended exposure to room or higher temperatures. Equilibrate all media 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 antibiotics to your formulation, a concentration of 10 mL/L is appropriate. Use Thermo Scientific HyClone Pen/Strep/Fungizone (SV30079.01).
- Discard unused medium after 8 weeks.

### Subculturing

1. In the laminar flow hood, remove spent medium from cell monolayer and discard.
2. Wash the monolayer with Thermo Scientific HyClone DPBS ES Qualified (SH30850.03) by adding 10 mL/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) or HyQTase (SV30030.01) at 3-5 mL/75 cm<sup>2</sup> flask and rock the flask to ensure that the entire monolayer is covered with the solution.
4. If using trypsin, incubate at 37°C until the hMSCs begin to detach (approximately 5 minutes). If using HyQTase, use at room temperature until the hMSCs begin to detach (approximately 5 minutes). Do not exceed 15 minutes. Care should be taken that the cells not be forced to detach prematurely, as this may result in clumping.
5. Add complete hMSC expansion medium (Table 1) in equal amounts to trypsin or HyQTase and pipette the cells up and down until the cells are dispersed into a single cell suspension.
6. To remove the trypsin or HyQTase, centrifuge cells for approximately 10 minutes at room temperature. Aseptically remove supernatant.
7. Resuspend the cell pellet in prewarmed complete hMSC expansion medium (Table 1) at approximately 5 mL/pellet from 75 cm<sup>2</sup> flask. Remove a small volume sample for counting.
8. Count the cells with a hemacytometer or cell counter and calculate cell count.
9. Seed new flasks at 5,000 to 6,000 cells/cm<sup>2</sup> by adding the appropriate volume of cell suspension into fresh pre-warmed complete hMSC expansion medium (Table 1).
10. Incubate cells at 37°C, 5% CO<sub>2</sub> and 90% humidity.
11. Perform a medium exchange 3-4 days after subculture, by replacing spent medium with equal volumes of fresh medium (Table 1). Cultures should be ready to subculture every 5-7 days.

### Related Protocols

- SC Protocol 00007 - Human Mesenchymal Stem Cell Protocol: Cryopreservation
- SC Protocol 00008 - Human Mesenchymal Stem Cells and Multipotent Cord Blood Unrestricted Somatic Stem Cell Protocol: Thawing and Plating

### References:

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

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