

Human Mesenchymal Stem Cell Protocol: Subculturing hMSC's

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. Any unused Thermo Scientific HyClone AdvanceSTEM™ Stem Cell Growth Supplement should be aliquoted and refrozen.

Subculturing

1. In the laminar flow hood, pipette spent medium from cell monolayer and discard spent medium.
2. Wash the monolayer with Thermo Scientific HyClone DPBS ES Qualified (SH30850.03) by adding 10 mL/75cm² 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) at 3-5 mL/75 cm² flask and rock the flask to ensure that the entire monolayer is covered with the trypsin solution.
4. Incubate at 37°C 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 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 prewarmed complete hMSC Expansion Medium (Table 1) at approximately 5 mL/pellet from 75 cm² 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/cm² 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% CO₂,

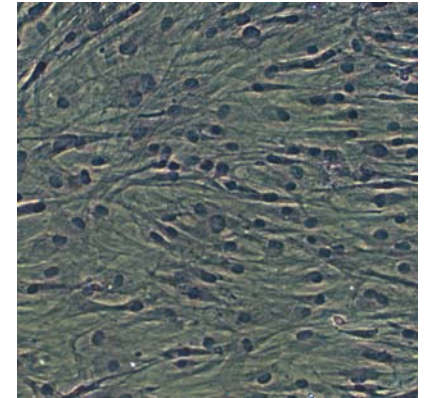


Figure 1: hMSCs cultured in Thermo Scientific HyClone AdvanceSTEM™ Mesenchymal Stem Cell Basal Medium supplemented with 10 percent Thermo Scientific HyClone AdvanceSTEM™ Stem Cell Growth Supplement.

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 6-7 days.

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.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

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