

Human Mesenchymal Stem Cell Protocol: Adipogenic Differentiation

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

Thermo Scientific HyClone AdvanceSTEM™ Adipogenic Differentiation Kit has been developed to support the Adipogenic differentiation of human Mesenchymal Stem Cells isolated from adipose tissue and bone marrow.

Notes: Please store all media at 2 - 8°C and avoid extended exposure to room or higher temperatures. Please equilibrate all media in a water bath set at 37°C before adding media to any cell culture.

Subculturing

1. In a laminar flow hood, pipette spent medium from cell monolayer and discard spent medium.
2. Wash the monolayer with Thermo Scientific HyClone ES-Qualified DPBS (SH30850.03) by adding 10mL/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 (SH30042.01) 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 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 Mesenchymal Stem Cell 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. Re-suspend the cell pellet in pre-warmed complete Mesenchymal Stem Cell Expansion Medium (Table 1) at approximately 5 mL/pellet for a 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. Plate on a fresh tissue culture dish at 80-90% confluency using complete Mesenchymal Stem Cell Expansion Medium (Table 1).
10. Let cells attach for a minimum

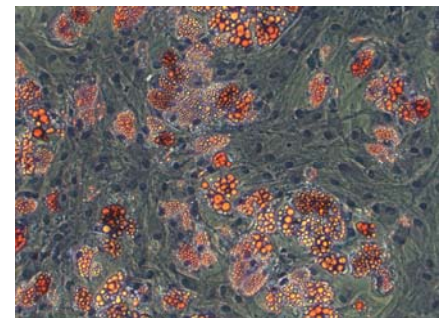


Figure 1: MSC cultured in Thermo Scientific HyClone complete AdvanceSTEM™ Adipogenic Differentiation Medium.

- of 24 hours or until normal morphology is seen.
11. Once the cells have attached and you have reached this level of confluency, withdraw the complete Mesenchymal Stem Cell Expansion Medium.
12. Rinse the monolayer with two rinses of DPBS.
13. Add complete Adipogenic Differentiation Medium (Table 2). For a 60 mm dish, about 7 ml is sufficient. Amount of medium will vary depending on the size of the culture dish being used.
14. Incubate the cells at 37°C, 5% CO₂, with humidity.
15. Withdraw all of the media every 3 days and replace with fresh, complete Adipogenic Differentiation Medium (Table 2). Adipogenesis can be seen as formation of lipid droplets within 7 days and peaks at 3 to 4 weeks.

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.

Table 2: Preparation of 500 mL complete Adipogenic Differentiation Kit

Thermo Scientific HyClone AdvanceSTEM™ Adipogenic Differentiation Kit (SH30876.KT)			
Brand	Amount for 500 mL	HyClone Product	Catalog #
Thermo Scientific	450 mL	AdvanceSTEM™ Adipogenic Differentiation Medium	SH30886.02 (450mL)
Thermo Scientific	50 mL	AdvanceSTEM Growth Supplement	SH30878.02 (50mL)

Note: 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 per liter is appropriate. Use Thermo Scientific HyClone Pen/Strep/Fungizone, SV30079.01

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