

# Human Mesenchymal Stem Cell Protocol: Adipogenic Differentiation

Protocol  
SC 00010

*For research use only*

## *Background*

Thermo Scientific HyClone AdvanceSTEM Adipogenic Differentiation Kit (SH30786.KT) has been developed to support the adipogenic differentiation of human mesenchymal stem cells isolated from adipose tissue and bone marrow. Adipogenic differentiation can be seen as formation of lipid droplets within 7 days and peaks at 3 to 4 weeks.

## Required Materials

- Thermo Scientific HyClone AdvanceSTEM™ Complete Mesenchymal Stem Cell Expansion Media
- Thermo Scientific HyClone AdvanceSTEM Complete Adipogenic Kit
- Thermo Scientific HyClone ES-Qualified DPBS (SH30850.03)
- Thermo Scientific HyClone Trypsin (SH30042.01)

## *Media Preparation*

Table 1: 500 mL Complete Thermo Scientific HyClone AdvanceSTEM hMSC Expansion Medium

Thermo Scientific HyClone Product	Volume (500mL 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.		

Table 2: 500 mL Complete Thermo Scientific HyClone AdvanceSTEM Adipogenic Kit (SH30786.KT)

Thermo Scientific HyClone Product	Volume (500 mL final)	Catalog Number
AdvanceSTEM Adipogenic Differentiation Medium	450 mL	SH30886.02 (450 mL)
AdvanceSTEM Cell Growth Supplement	50 mL	SH30878.02 (50 mL)
Store at 2-8°C. Discard unused medium after 2 weeks.		

**Note:** Antibiotics should not be used as an alternative to proper aseptic technique. However, should you prefer to add antibiotics to your medium use [1%] of Thermo Scientific HyClone Pen/Strep (SV30079.01).

*Protocol*

1. In a laminar flow hood, pipette spent medium from the cell monolayer and discard.
2. Wash the monolayer with Thermo Scientific HyClone ES-Qualified DPBS (SH30850.03). 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 Thermo Scientific HyClone AdvanceSTEM Mesenchymal Stem Cell 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 AdvanceSTEM Mesenchymal Stem Cell Expansion Media. Remove a small sample volume for counting.
7. Count the cells with a hemacytometer or cell counter and calculate desired number.
8. Plate on a fresh tissue culture treated dish at 80-90% confluency using complete HyClone AdvanceSTEM Mesenchymal Stem Cell Expansion Medium (Table 1). Incubate at 37°C and 5% CO<sub>2</sub>.
9. When the cells have attached for a minimum of 24 hours or until normal morphology is seen, remove the medium.
10. Add fresh complete AdvanceSTEM Adipogenic Differentiation Medium (Table 2).
11. Every 3 days remove all media and replace with fresh complete AdvanceSTEM Adipogenic Differentiation Medium (Table 2).

*Related Protocols*

- SC Protocol 00009 - Human Mesenchymal Stem Cell Protocol: Sub Culturing hMSCs
- SC Protocol 00011 - Human Mesenchymal Stem Cell Protocol: Oil Red O Staining of Adipogenic Cultures

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