

# Human Mesenchymal Stem Cell Protocol: Osteogenic Differentiation

Protocol  
SC 00013

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

*For research use only*

## *Background*

Thermo Scientific HyClone Osteogenic Differentiation Kit has been designed to support the osteogenic differentiation of human mesenchymal stem cells isolated from adipose tissue and multipotent cord blood unrestricted somatic stem cells.

## Required Materials

- Adipose or multipotent cord blood unrestricted somatic stem cells
- Complete hMSC expansion medium (see Table 1)
- Complete osteogenic differentiation medium (see Table 2)
- Thermo Scientific HyClone ES Qualified DPBS (SH30850.03)
- Thermo Scientific HyClone Trypsin (SH30042.01) or HyQTase (SV30030.01)
- General cell culture supplies

## *Media Preparation*

Table 1: Complete hMSC Expansion Medium

Thermo Scientific HyClone AdvanceSTEM Mesenchymal Stem Cell Expansion Kit (SH30875.KT)		
Thermo Scientific HyClone Product	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: Complete Osteogenic Differentiation Medium

Thermo Scientific HyClone AdvanceSTEM Mesenchymal Stem Cell Expansion Kit (SH30875.KT)		
Thermo Scientific HyClone Product	Volume (500 mL final)	Catalog Number
AdvanceSTEM Osteogenic Differentiation Medium	450 mL	SH30881.02 (450 mL)
AdvanceSTEM Stem Cell Growth Supplement	50 mL	SH30878.02 (50 mL)

## *General Considerations*

- 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.

### *Osteogenic Differentiation Protocol*

1. In a laminar flow hood, pipette spent medium from cell monolayer and discard spent medium.
2. Wash the monolayer with HyClone ES Qualified DPBS (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 Trypsin or HyQTase 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 warmed complete mesenchymal stem cell expansion medium (Table 1) at approximately 5 mL/pellet for a 75cm<sup>2</sup> flask. Remove a small 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 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 osteogenic differentiation medium (Table 2). For a 60mm 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 and 5% CO<sub>2</sub> with humidity.
15. Withdraw all of the media every 3 days and replace with fresh, complete osteogenic differentiation medium (Table 2).
16. Osteogenesis takes approximately 21-28 days and can be seen by the formation of osteoblasts, which are tightly packed and linear in shape. In addition, mineralized matrix can be detected by a variety of staining protocols.

### *Suggested Staining Protocols*

- Alkaline phosphatase
- Alizarin Red S Staining Protocol for Calcium

### *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
- SC Protocol 00009 - Human Mesenchymal Stem Cell Protocol: Subculturing hMSCs

### *References:*

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