

# Human Mesenchymal Stem Cell Protocol: Osteogenic Differentiation

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

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.

*Note: 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.*

## Thawing Cells

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/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 (SH30042.01) at 3-5 mL/75 cm<sup>2</sup> flask and rock the flask to

ensure that the entire monolayer is covered with the 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 not to force the cells 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. Resuspend the cell pellet in pre-warmed **complete** Mesenchymal Stem Cell Expansion Medium (Table 1) at approximately 5 mL/pellet for a 75 cm<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

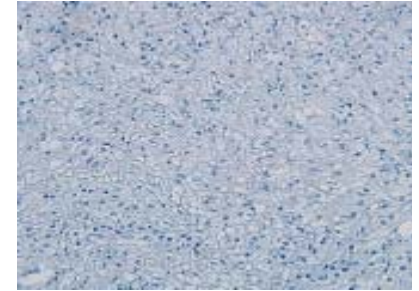


Figure 1: Cells stained for cartilage using toluidine blue staining

**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 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% 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.

Table 1: Preparation of 500 mL complete hMSC Expansion Medium

Thermo Scientific HyClone AdvanceSTEM™ Mesenchymal Stem Cell Expansion Kit (SH30875.KT)			
Brand	Amount for 5 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 Osteogenic Differentiation Medium**

<b>Thermo Scientific HyClone AdvanceSTEM™ Osteogenic Differentiation Kit (SH30877.KT)</b>			
<b>Brand</b>	<b>Amount for 500 mL</b>	<b>HyClone Product</b>	<b>Catalog #</b>
<b>Thermo Scientific</b>	<b>450 mL</b>	<b>AdvanceSTEM Osteogenic Differentiation Medium</b>	<b>SH30881.02 (450 mL)</b>
<b>Thermo Scientific</b>	<b>50 mL</b>	<b>AdvanceSTEM™ Stem Cell Growth Supplement</b>	<b>SH30878.02 (50 mL)</b>

*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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