

Human Mesenchymal Stem Cell Protocol: Neural Differentiation

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

Thermo Scientific HyClone AdvanceSTEM Neural Differentiation Kit has been developed to support a variety of human somatic stem cells into neural cells. These include: Adipose-Derived MSCs, Bone Marrow Derived MSCs 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/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 not be forced to detach prematurely, as this may result in clumping.

5. Add **complete** Thermo Scientific HyClone AdvanceSTEM™ Mesenchymal Stem Cell Expansion Media (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 the supernatant.
7. Resuspend the cell pellet in pre-warmed **complete** Thermo Scientific HyClone AdvanceSTEM Neural Differentiation Media (Table 2) at approximately 5 mL/pellet for a 75 cm² flask. Remove a small sample for counting.
8. Count the cells with a hemacytometer or cell counter and

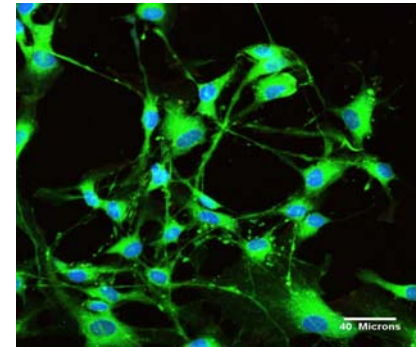


Figure 1: MCBUSSCs cultured in Thermo Scientific HyClone complete AdvanceSTEM™ Neural Differentiation Kit.

- calculate cell count.
9. Plate on a fresh tissue culture dish at 30% confluency (approximately 2500 cells/cm²) using **complete** Mesenchymal Stem Cell Expansion Media (Table 1)
10. Let cells attach for 24 hours or until normal morphology is seen. Once the cells have attached and you have reached this level of confluency, withdraw your **complete** Mesenchymal Stem Cell Expansion Media (Table 1).
11. Rinse the monolayer with two rinses of Dulbecco's phosphate buffered saline, (SH30850.03).
12. Add **complete** Neural Differentiation Media (Table 2). For a 60 mm dish, about 7 ml is sufficient. Add more or less media depending on the size of dish.
13. Incubate the cells at 37°C, 5% CO₂, with humidity.
14. Every 48 hours withdraw and add new **complete** Neural Differentiation Medium (Table 2). Neural differentiation can be seen as formation of neuron like cells within 24 hours and peaking at 72 hours.
15. To maintain cells in a differentiated state, add additional Neural Differentiation Medium every 48 hours.

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 Neural Differentiation Medium

Thermo Scientific HyClone AdvanceSTEM™ Neural Differentiation Kit (SH30892.KT)			
Brand	Amount for 500 mL	HyClone Product	Catalog #
Thermo Scientific	450 mL	AdvanceSTEM Neural Differentiation Medium	SH30893.02 (450 mL)
Thermo Scientific	50 mL	AdvanceSTEM™ Stem Cell Growth Supplement	SH30878.02 (50 mL)

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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