

Human Mesenchymal Stem Cell Protocol: Neural differentiation

Protocol
SC 00014

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

For research use only

Background

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.

Required Materials

- Adipose, Bone Marrow or Multipotent Cord Blood Unrestricted Somatic Stem Cells
- Complete hMSC expansion medium (see Table 1)
- Complete neural differentiation medium (see Table 2)
- Thermo Scientific HyClone ES Qualified DPBS (SH30850.03)
- Thermo Scientific HyClone HyQTase (SV30030.01) or Trypsin (SH30042.01)
- 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	Amount for 500 mL	Catalog Number
AdvanceSTEM™ Mesenchymal Stem Cell Basal Medium	450 mL	SH30879.02
AdvanceSTEM Stem Cell Growth Supplement	50 mL	SH30878.02

Table 2: Complete Neural Differentiation Medium

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

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.

Neural Differentiation Protocol

1. In a laminar flow hood, pipette spent medium from cell monolayer and discard spent medium.
2. Wash the monolayer with DPBS by adding 10 mL / 75 cm² to the flask, being careful not to disturb the monolayer. Rock the flask bank and forth. Remove the DPBS from the monolayer and discard.
3. Add HyQTase or Trypsin 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 ambient conditions (between 15°C and 25°C) for 10 minutes or until the cells are dissociated from the flask and free of clumps if using HyQTase. Incubate at 37°C for 10 minutes or until the cells are dissociated from the flask and free of clumps if using trypsin. Do not exceed 15 minutes with trypsin. Care should be taken that the cells not be forced to detach prematurely, as this may result in clumping.
5. Add complete hMSC expansion media (Table 1) in equal amounts to dissociation solution and pipette the cells up and down until the cells are dispersed into a single cell suspension.
6. To remove Trypsin, centrifuge cells at approximately 200 x g for 10 minutes at room temperature. Aseptically remove the supernatant. If using HyQTase, removal by centrifugation is not necessary, proceed to step 8.
7. Re-suspend the cell pellet in pre-warmed complete hMSC expansion media (Table 1) at approximately 5 mL/pellet for a 75 cm² flask. Remove a small sample for counting.
8. Count the cells with a hemacytometer or other cell counting device and calculate the cell concentration.
9. Plate on a fresh tissue culture dish at 30% confluency (approximately 2500 cells/ cm²) using complete hMSC Expansion Medium (Table 1).
10. Let cells attach for 24 hours or until normal morphology is seen. Once the cells have attached and reached 30% confluency, withdraw the complete mesenchymal stem cell expansion media.
11. Rinse the monolayer twice with DPBS.
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 the culture vessel.
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.

Related Protocols:

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

References:

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