Neural Differentiation Media

NEU.D.Media-450

Media Usage Protocol:

Neural Differentiation Media is designed to be used with Human Adipose Derived Mesenchymal Stem Cells, Human Bone Marrow Mesenchymal Stem Cells and Human Multipotent Cord Blood Unrestricted Somatic Stem Cells, all of which are available separately. When used as directed, this media will support differentiation of these stem cells into neuron like cells. The following is the recommended protocol for the usage of this media

Note: Once complete media has been formulated, it should be stored at 4 ℃. Avoid extended exposure of the media to room or higher temperatures. Media should be equilibrated in a water bath set at 37 ℃ before adding to any cell culture.

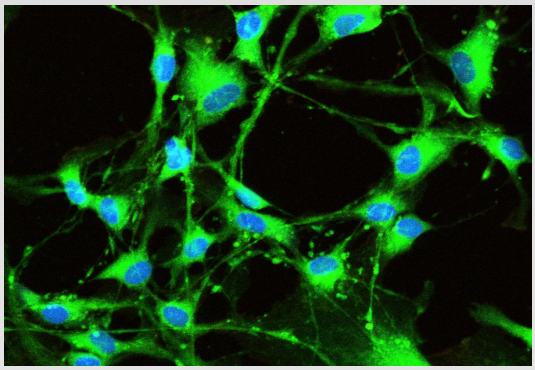

Figure 1: The figure shows neuron like cells differentiated from adipose derived mesenchymal stem cells after 24 hours in CET's Neural Differentiation Media. Cells were stained with Topro-3 and mitotracker dyes.

Note: Antibiotics/ antimycotics should not be used as an alternative to proper aseptic technique.

Additional Reagents Needed

1. Fetal Bovine Serum, High Grade or Characterized. Store in aliquots of 50 mL at –20°C.
2. Penicillin/Streptomycin/Amphotericin B solution, 100X or Penicillin/Streptomycin solution, 100X. These solutions should be portioned in 5 mL aliquots, stored at -20℃ and never freeze/thawed. Although antimycotics are not absolutely necessary, CET highly recommends their usage for long term cell culture.
3. A monolayer of actively growing adipose derived mesenchymal stem cells, bone marrow derived mesenchymal stem cells or human multipotent cord blood unrestricted somatic stem cells.

Preparing Adipose, Bone Marrow Derived Mesenchymal Stem Cells or Multipotent Unrestricted Somatic Stem Cells

1. In a laminar flow hood, pipette spent complete Mesenchymal Stem Cell/Multipotent Unrestricted Somatic Stem Cell Expansion Media from the cell monolayer and discard.
2. Wash the monolayer with Dulbecco's Phosphate Buffered Saline (DPBS). Use 10 mL/T-75 flask. Rock the flask gently, then remove the DPBS and discard.
3. Add 0.25% Trypsin/EDTA solution at 5 mL/T-75 flask. 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 must be taken that the cells are not forced to detach prematurely, as this may result in clumping.
4. Inactivate the trypsin by adding at least an equal volume of complete Mesenchymal Stem Cell/Unrestricted Somatic Stem Cell Expansion Media. Pipette the cells up and down to further separate into a single cell suspension.
5. Centrifuge the cells at 200 x g for 10 minutes. Carefully remove supernatant.
6. Resuspend the cells in 10 mL complete Mesenchymal Stem Cell/Unrestricted Somatic Stem Cell Expansion Media.
7. Plate the cells on a fresh tissue culture dish at 30% confluency (approximately 2500 cells/cm^2) using complete Mesenchymal Stem Cell/Unrestricted Somatic Stem Cell Expansion Media. It is critical not to exceed this cell density since the efficiency of neural differentiation will drop exponentially with higher densities.
8. 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/Unrestricted Somatic Stem Cell Expansion Media.
9. Rinse the monolayer twice with Dulbecco's Phosphate Buffered saline.

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Neural Differentiation

1. Add complete Neural Differentiation Media. For a 60 mm dish, about 7 mL is sufficient. Add more or less media depending on the size of the culture vessel.
2. Incubate the cells at 37°C, 5% CO_2, with humidity.
3. Every 48 hours withdraw and add new complete Neural Differentiation Media. Neural differentiation can be seen as formation of neuron like cells within 24 hours and peaking at 72 hours.
4. To maintain cells in a differentiated state, add additional complete Neural Differentiation Media every 48 hours.

Key References:

1. Exp Neurol. 2007 Oct;207(2):267-74. Epub 2007 Aug 2.
Adipose-derived stem cells differentiate into a Schwann cell phenotype and promote neurite outgrowth in vitro. Kingham PJ, Kalbermatten DF, Mahay D, Armstrong SJ, Wiberg M, Terenghi G.

2. Plast Reconstr Surg. 2005 Oct;116(5):1453-60.
The potential of adipose-derived adult stem cells as a source of neuronal progenitor cells. Kokai LE, Rubin JP, Marra KG.

Certificate of Analysis

All hematopoietic, mesenchymal and multipotent stem cells are evaluated by flow cytometry for specific stem cell markers. All other cells are evaluated either by staining, method of isolation or traditional molecular biology techniques. Data is available upon request.

All growth and differentiation media are evaluated by conducting assays to make sure cells either grow or differentiate as indicated on the media label. Data is available upon request.

All cells are tested for HIV-1, HIV-2, Hepatitis B and Hepatitis C using sensitive PCR based assays. All cells test negative for these viruses. However, all human cells must be used in accordance with established laboratory safety procedures and only under the supervision of trained personnel.

Table 1: Preparation of 500 mL complete Neural Differentiation Media

Brand	Amount For 500 mL	CET Product	Catalog #
CET	450 mL	CET Neural Differentiation Media	NEU.D.Media-450
Any	50 mL	Fetal Bovine Serum	Refer to Manufacturer's Catalog Number

Store at 4°C.

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