Data Sheet

MN9D Mouse Dopaminergic Neuronal Cell Line

Immortalized Hybrid Cell Line

SCC281

Pack Size ≥ 1x10^6 viable cells/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

The mechanisms by which central neurons develop axons and establish functional connections with their target cells as well as the role of such connections in cell survival are important developmental neurobiology questions studied using *in vitro* cell-reaggregation systems, both for central dopaminergic and cholinergic neurons. Hybrid clonal cells facilitate the study of trophic interactions between mesencephalic dopaminergic neurons and their target cells.

Source

The hybrid MN9D immortalized dopaminergic neuronal cell line was generated by fusion between rostral mesencephalic neurons from a 14-day-old C57BL/6J mouse embryo and N18TG2 neuroblastoma cells, a sympathetic nervous system cancer of A/Jax background.[1] MN9D cells produce dopamine (DA) and express tyrosine hydroxylase (TH) as well as aromatic amino acid decarboxylase (AADC).[2] MN9D cells readily aggregate with one another or other embryonic brain cells[2] and can be transfected using calcium phosphate precipitation[3] or Lipofectamine.[4]

Undifferentiated or differentiated MN9D cells are extensively used to model dopaminergic neurons and to test mechanisms and potential therapeutics relevant to the loss of DA neurons in Parkinson's disease. Differentiated with butyric acid or glial cell line-derived neurotrophic factor (GDNF) followed by butyric acid, MN9D cells only partially recapitulate the electrophysiological properties of midbrain DA neurons. Optimizing MN9D differentiation further using one or a combination of growth or other factors may yield an improved model system for Parkinson's disease studies *in vitro*.[5]

Short Tandem Repeat (STR profile)

M1-1: 11, 17	M8-1: 16, 17
M1-2: 17, 19	M11-2: 15, 16
M2-1: 16	M12-1: 16, 17
M3-2: 13, 14	M13-1: 16.2, 17.2, 18.2
M4-2: 20.3, 22.3	M15-3: 22.3, 23.3
M5-5: 15, 17	M17-2: 15, 16
M6-4: 18, 20	M18-3: 16, 22
M6-7: 12, 17	M19-2: 12, 13
M7-1: 25.2	MX-1: 25, 28, 29

Quality Control Testing

- MN9D cells are verified to be of mouse origin and negative for rat, Chinese hamster, Golden Syrian hamster, human, and non-human primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious diseases by a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

MN9D cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting cell marker expression and functionality. Cells should not be expanded beyond p20.

Representative Data

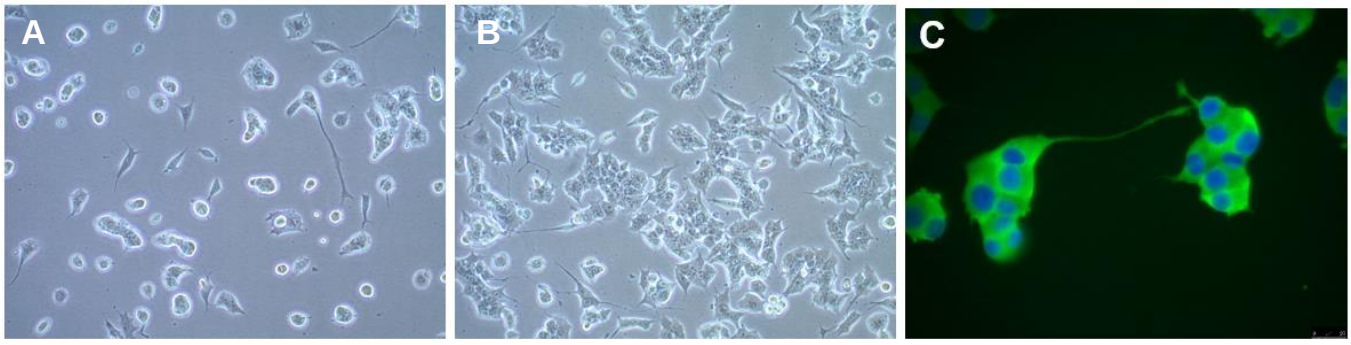

Figure 1. MN9D cells tend to grow in clusters. Bright-field images of MN9D culture one (**A**) and two days (**B**) after thaw. MN9D cells express tyrosine hydroxylase (**C**).

Protocols

ECM-Coating the Cultureware

MN9D cells grow on cultureware surfaces coated with poly-L-Lysine.

1. Cover the growth area of cultureware with 1 mg/mL poly-L-Lysine solution (for example, P1274), using 3 mL for T25, 6 mL for T75, and 15 mL for T225 flasks, and incubate at 37 °C for at least 1 hour. Flasks may also be coated overnight.
2. Aspirate the coating solution, rinse the surface thrice with 15 mL sterile DI water, and aspirate dry.
3. Allow flasks to dry for at least 2-3 hours at room temperature before introducing cells and medium.

Thawing the Cells

Do not thaw the cells until the recommended medium and coated cultureware are on hand.

1. Cells are thawed and expanded in MN9D Expansion Medium comprising High Glucose DMEM containing L-glutamine (for example,. D5796), and 10% FBS (for example, ES-009-B).
2. Remove the vial of frozen MN9D cells from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1- or 2-mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of MN9D Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 15 mL of MN9D Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing the Cells

1. MN9D cells tend to grow in small clusters. MN9D cells should be passaged at ~ 80% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of MN9D cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of Accutase® and incubate in a 37 °C incubator for 3-5 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5-7 mL of MN9D Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of MN9D Expansion Medium to the conical tube and resuspend the cells thoroughly. Cell clumps may be broken up by extensive trituration using a 5 mL serological pipette.

    IMPORTANT: Do not vortex the cells.

11. Count the number of cells using a hemocytometer.
12. Plate the cells to the desired density into poly-L-Lysine coated cultureware. Typical split ratio is 1:6.

Differentiating the Cells

Differentiated MN9D cells no longer divide and develop longer processes as seen in primary neurons. Differentiation is often dependent on plating density and takes 7-10 days. Not 100% of the cells differentiate.

1. Plate 250,000 cells in a 100 mm plate treated with poly-L-Lysine in MN9D Expansion Medium supplemented with one of the following:
   - 1 mM n-butyrate (B5887),
   - 1 mM dibutyryl cAMP (B0260), or
   - 10 µM forskolin (F6886), dissolved in DMSO
2. Change the differentiation medium every 2-3 days.

Cryopreservation of the Cells

MN9D Mouse Dopaminergic Neuronal Cells may be frozen in MN9D Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

References

1. Choi HK, Won LA, Kontur PJ, Hammond DN, Fox AP, Wainer BH, Hoffmann PC, Heller A. (1991) Immortalization of embryonic mesencephalic dopaminergic neurons by somatic cell fusion. Brain Res 552(1):67–76.
2. Choi HK, Won L, Roback JD, Wainer BH, Heller A. (1992) Specific modulation of dopamine expression in neuronal hybrid cells by primary cells from different brain regions. Proc Natl Acad Sci USA 89(19):8943–8947.
3. Perez RG, Waymire JC, Lin E, Liu JJ, Guo F, Zigmond MJ. (2002) A role for alpha-synuclein in the regulation of dopamine biosynthesis. J Neurosci 22(8):3090-3099.
4. Cavanaugh JE, Jaumotte JD, Lakoski JM, Zigmond MJ. (2006) Neuroprotective role of ERK1/2 and ERK5 in a dopaminergic cell line under basal conditions and in response to oxidative stress. J Neurosci Res 84(6):1367-1375.
5. Rick CE, Ebert A , Virag T, Bohn MC, Surmeier DJ. (2006) Differentiated dopaminergic MN9D cells only partially recapitulate the electrophysiological properties of midbrain dopaminergic neurons. Dev Neurosci 28(6):528-537.

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