Data Sheet

N27 Rat Dopaminergic Neural Cell Line

Immortalized Cell Line

SCC048

Pack Size: ≥ 1 x 10^6 cells/vial

Store in Liquid Nitrogen

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Background

Parkinson's disease is a neurodegenerative disorder of the central nervous system that affects more than 6 million people worldwide. The motor symptoms of Parkinson's disease result from the death of dopamine generating cells in the substantia nigra, a region of the midbrain. Numerous efforts have been made to slow the loss or replace these dopamine producing neurons *in vivo*.

The N27 Rat Dopaminergic Neuron Cell Line (N27 Cells) was harvested from E12 rat mesencephalic tissue and was transfected with SV40 to immortalize the cell line. The N27 cell line, when injected into the striata of 6-hydroxydopamine-lesioned rats (an animal model of PD) caused a time-dependent improvement in neurological deficits. This immortalized cell line has been carefully characterized in studies of dopamine biosynthesis, neurotoxicity and used as a dopaminergic neuron model for *in vitro* and *in vivo* studies. This product contains genetically modified organisms.

Quality Control Testing

Cell Count: ≥ 1 x 10^6 cells/vial

Mycoplasma: Negative

Proliferation Upon Thaw: Pass

Storage and Handling

The N27 Cells should be stored in liquid nitrogen. The cells can be passage for at least 10 passages without significantly affecting the cell marker expression and functionality.

Presentation

The N27 Cells are supplied frozen in 10% DMSO and 90% expansion medium.

Protocols

Thawing and Subculture

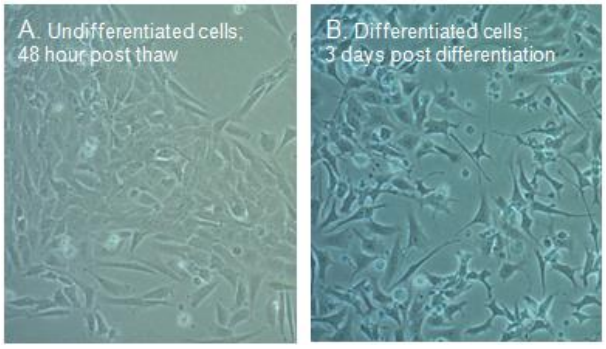
Prior to thawing cells, prepare culture medium according to Table 1. It is recommended to count cell and perform viability test upon thawing and passage.

Retrieve a vial of the N27 Rat Dopaminergic Neuron Cell Line and thaw in a 37 °C water bath. Do not completely thaw; make sure that there is still a sliver of frozen cells in the vial. Spray the vial with 70% isopropyl alcohol before placing the cells in the biological safety cabinet.

1. Add 9 mL culture medium to a sterile 15 mL conical tube.
2. Slowly transfer the thawed cells into the 15 mL conical tube.
3. Re-suspend the cells by inverting the conical tube.
4. Centrifuge the cell suspensions for 5 minutes at 1000 rpm.
5. Aspirate the supernatant and re-suspend the pellets in total volume of 10 mL of rat dopaminergic neuronal line culture medium.
6. Plate cells to T75 flask, culture cells at 5% CO_2, 37 °C tissue culture incubator.
7. Change medium every day.
8. Cells are ready to be passage upon 70-90% confluency.
9. Rinse cells with 10 mL PBS.
10. Remove PBS and add 3 mL 0.05% Trypsin-EDTA (SM-2002-C), carefully rock the plate to cover the entire surface with Trypsin solution.
11. Incubate at 37 °C for 3-5 minutes, tap the flask to facilitate the dissociation of cells.
12. Add 10 mL culture medium to flask and transfer cell suspension to a sterile 15 mL conical tube.
13. Centrifuge 1000 RPM for 5 minutes.
14. Remove supernatant and resuspend cells with 5 mL culture medium. Split 1:5 to 1:10 into a new flask.

Table 1. N27 Rat Dopaminergic Neuron Culture Medium

Component	% (v/v)	Catalogue Number
RPMI1640	90%	SLM-140-B
ES Cell Qualified FBS	10%	ES-009-B
Penicillin-Streptomycin (Optional)	1%	TMS-AB2-C
L-Glutamine Solution (100x)	1%	TMS-002-C



Differentiation

1. Coat tissue culture plate with 0.01% poly-L-Lysine (A005-C) at room temperature for 30 minutes.
2. Plate cells at 1-2 x 10^4 cells/cm^2 in culture medium.
3. After 24-hour post seeding, change medium to culture medium with 60 µg/mL DHEA (D063) and 2 mg/mL dibutyryl cyclic AMP (28745-25 mg).
4. Differentiate cells for 3 days at 37 °C.

References

N27 Rat Dopaminergic Neuron Characterization

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Neurotoxicity Assay and Oxidative Stress Assay

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Dopamine Signaling Pathway:

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