N27 Rat Dopaminergic Neural Cell Line

Immortalized Cell Line

Cat. # SCC048

pack size: ≥ 1X10^6 cells/vial

Store in Liquid Nitrogen

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION
THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS.

Certificate of Analysis

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 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.

Presentation

Product is supplied frozen in 10% DMSO and 90% expansion medium.

Quality Control Assay

Cell Count: ≥ 1X10^6 cells/vial

Mycoplasma: Negative

Proliferation Upon Thaw: Pass

Storage and Handling

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.

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.

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

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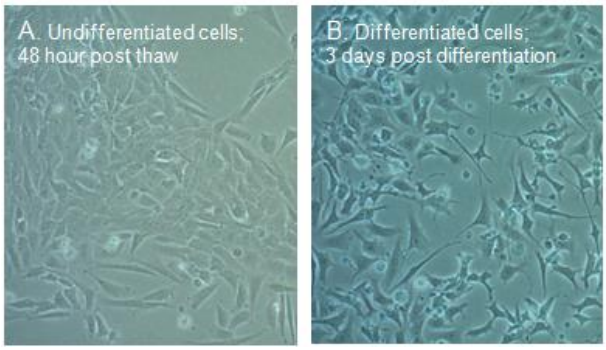
SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

Table 1. N27 Rat Dopaminergic Neuron Culture Medium

Component	*Millipore Cat. No.*	*% (v/v)*
RPMI1640	*SLM-140-B*	*90%*
ES Cell Qualified FBS	*ES-009-B*	*10%*
Penicillin-Streptomycin (Optional)	*TMS-AB2-C*	*1%*
L-Glutamine Solution (100x)	*TMS-002-C*	*1%*



Differentiation

1. Coat tissue culture plate with 0.01% poly-L-Lysine (Cat No. A005-C) at room temp for 30 min.
2. Plate cells at 1-2x10^4 cells/cm^2 in culture medium.
3. 24 hour post seeding, change medium to culture medium with 60μg/mL DHEA (Sigma D063) and 2mg/mL dibutyryl cyclic AMP (Cat. No. 28745-25mg).
4. Differentiate cells for 3 days at 37°C.

References

N27 Rat dopaminergic neuron characterization:

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