N27-A Rat Dopaminergic Neural Cell Line

Neural Cell Line

Cat. # SCC196

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.
THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS.

Data Sheet

Background

Parkinson's disease is characterized by the death of dopaminergic neurons in the substantia nigra of the brain.[1] In vitro models are critical for understanding the molecular pathology of Parkinson's disease. The N27 immortalized rat dopaminergic cell line has been widely utilized in Parkinson's research for over 20 years. However long-term passaging has led the N27 cell line to become a mixture of cell types with widely variable expression of dopaminergic neural markers such as tyrosine hydroxylase (TH). N27-A is a clonal derivative of N27 characterized by consistently high expression of the dopaminergic markers TH, dopamine transporter (DAT), and Tuj1.[3] N27-A cells express the dopaminergic neuron transcription factors *Nurr1, En1, FoxA2*, and *Pitx3* as well as the monoamine transporter VMAT2, but do not express dopamine-beta-hydroxylase (DβH), the enzyme that converts dopamine to norepinephrine. N27-A cells exhibit higher sensitivity to neurotoxins as compared to parental N27 cells, and release dopamine under both basal and depolarization conditions. These features make the N27-A cell line an improved model for Parkinson's disease research.

Source

The N27-A cell line is a clonal derivative of the parental N27 cell line selected for high expression of dopaminergic neural markers. The parental cell line was originally isolated from dopaminergic neurons from an embryonic day 14 rat mesencephalon and immortalized with SV40 large T antigen.[2]

Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Mouse/Rat Comprehensive CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of rat origin and negative for inter-species contamination from mouse, chinese hamster, Golden Syrian hamster, human and non-human primate (NHP) as assessed by a Contamination CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Storage & Handling

N27-A Rat Dopaminergic Neural Cell Line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

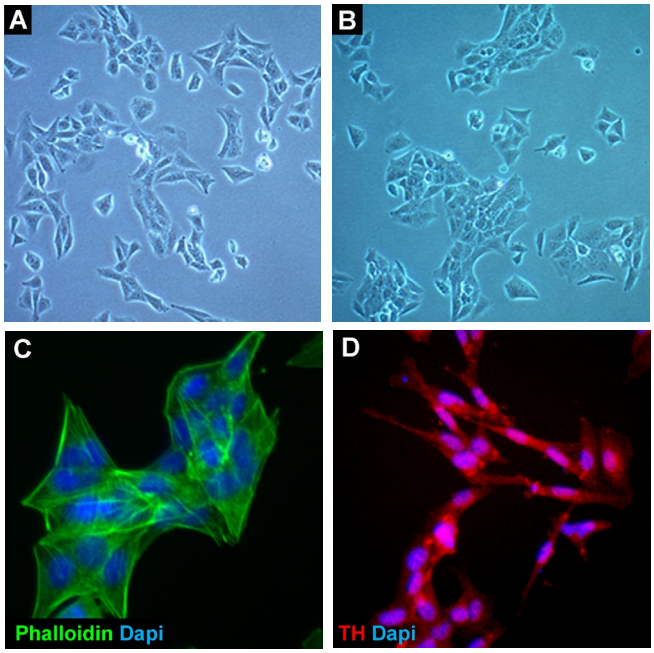

Figure 1. N27-A cells one (A, 10X magnification) and two (B, 10X magnification) days after thawing in a T75 flask. Cells express actin (C, Phalloidin; Sigma P5282) and tyrosine hydroxylase (D, Millipore MAB318-AF488).

References

1. Dexter DT, Jenner P (2013). *Free Radic Biol Med* 62: 132-144.
2. Clarkson ED, Rosa FG, Edwards-Prasad J, Weiland DA, Witta SE, Freed CR, Prasad KN (1998) *Proc Natl Acad Sci USA* 95(3): 1265-1270.
3. Gao L, Zhou W, Symmes B, Freed CR (2016) *PLoS One* 11(8): e0160847.

Rev.1.0/2019-03-19/SCC196/VC

Protocols

Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue cultureware surfaces without any additional coating.

   N27-A Expansion Medium: Cells are thawed and expanded in RPMI-1640 (Sigma Cat. No. R0883) supplemented with 2 mM L-Glutamine (Cat. No. TMS-002-C), and 10% FBS (Cat. No. ES-009-B).
2. Remove the vial of frozen N27-A 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 N27-A 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 N27-A 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 Cells

1. Do not allow the cells to grow to confluency. N27-A cells should be passaged at ~80-85% confluence.
2. Carefully remove the medium from the T75 tissue culture flask containing the N27-A cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
3. Apply 5-7 mL of Accutase and incubate in a 37°C incubator for 3-5 minutes.
4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
5. Add 5-7 mL of N27-A Expansion Medium to the plate.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
7. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
8. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
9. Apply 2-5 mL of N27-A Expansion Medium to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
10. Count the number of cells using a hemocytometer.
11. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of Cells

N27-A rat dopaminergic neural cell line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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