Sigma-Aldrich®

Data Sheet

N2A Psen1/Psen2 Cell Line

Engineered Cell Line

Cat. # SCC297

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 N2A Psen1/Psen2 cell line is a model for studies of presenilin function in the pathology of familial Alzheimer's Disease. Early-onset Alzheimer's disease (AD), also termed autosomal dominant AD with early onset (ADAD), is a devastating neurodegenerative disease that is strongly linked to familial inheritance. The main cause of early-onset/familial AD is autosomal dominant mutations in *APP* (amyloid precursor protein), *PSEN1* (presenilin-1) or *PSEN2* (presenilin-2).[1] As catalytic subunits of γ-secretase complex, which cleaves APP to form amyloid beta peptides, Psen1 and Psen2 play significant roles in the formation of amyloid plaques. Variations in *PSEN1/2* are frequently present in familial early-onset AD cases but their functional significance is unknown. Unraveling the functions of presenilin proteins is thus key to understanding the molecular pathology of AD.

The N2A Psen1/2 cell line was derived from CRISPR/Cas9-editing of the mouse N2A (Neuro2a) neuroblastoma cell line.[2] The N2A Psen1/2 cell line has been validated by functional and genetic assays to confirm deletion of Psen1/2.[2] N2A Psen1/2 cells express the neural markers glial fibrillary acid protein (GFAP) and MAP2, in common with the parental N2A cell line.[3] The N2A Psen1/2 cell line is a unique tool for the assessment of the contribution of *PSEN* mutations to AD pathology and widens the path to targeted therapies for AD and related neurodegenerative diseases.

Source

The N2A Psen1/2 cell line was derived via CRISPR/Cas9-editing of the parental N2A mouse neuroblastoma cell line.[2] The N2A (Neuro2a) parental cell line was derived from neuroblastoma stem cells of a male strain A/J mouse.[4]

Short Tandem Repeat (STR Profile)

Marker	Alleles	Marker	Alleles
M18-3:	22	M2-1:	16
M4-2:	21.3, 22.3	M15-3:	21.3, 22.3, 23.3, 24.3
M6-7:	12	M6-4:	18, 20
M19-2:	12	M11-2:	15
M1-2:	17, 18	M17-2:	16, 17
M7-1:	25.2	M12-1:	16
M1-1:	11	M5-5:	15, 17
M3-2:	13, 14	MX-1:	26, 27
M8-1:	16, 17	M13-1:	16.2, 17.2

Cell lines are inherently genetically unstable. Instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the US and Canada.

Submit your published journal article and earn credit toward future EMD Millipore purchases. Visit www.emdmillipore.com/publicationrewards to learn more!

EMD Millipore Corporation, 28820 Single Oak Drive, Temecula, CA 92590, USA 1-800-437-7500

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures. Not for human or animal consumption. Purchase of this Product does not include any right to resell or transfer, either as a stand-alone product or as a component of another product. Any use of this Product for purposes other than research is strictly prohibited. Chemicon®, Upstate®, and all other trademarks, unless specifically identified above in the text as belonging to a third party, are owned by Merck KGaA, Darmstadt, Germany. Copyright ©2008-2021 Merck KGaA, Darmstadt, Germany. All rights reserved. 20306518; Version 2.0

Quality Control Testing

- N2A Psen1/Psen2 cells are verified to be of mouse origin and negative for human, rat, Chinese hamster, Golden Syrian hamster, 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 against a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

N2A Psen1/Psen2 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 the cell marker expression and functionality.

Representative Data

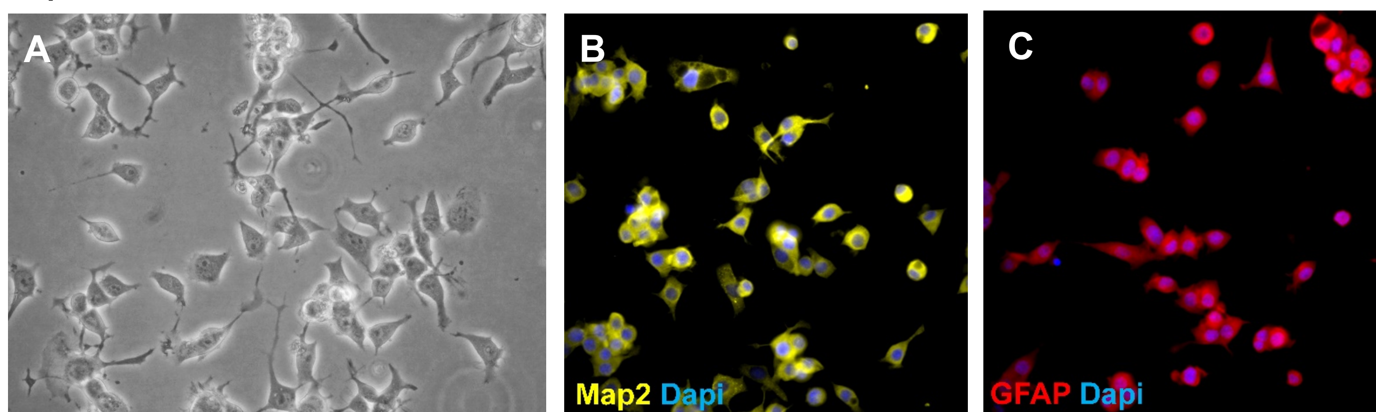

Figure 1. Bright-field image of N2A Psen1/2 cells two days (**A**) after thaw. Cells express neural markers Map2 (**B**, Sigma AB5622) and GFAP (**C**, Sigma AB5541).

Protocols

Thawing the Cells

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

   Cells are thawed and expanded in N2A Psen1/2 Expansion Medium comprising MEM medium (Sigma M2279) containing 10% FBS (e.g. Sigma ES-009-B), 10 mM HEPES (Sigma TMS-003-C) and 2 mM L-Glutamine (Sigma TMS-002-C).
2. Remove the vial of frozen N2A Psen1/2 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 N2A Psen1/2 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 N2A Psen1/2 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. Do not allow the cells to grow to confluency. N2A Psen1/2 cells should be passaged at ~70-80% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of HEK293 FUS KO 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 N2A Psen1/2 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 N2A Psen1/2 Expansion Medium to the conical tube and resuspend the cells thoroughly. Large cell clumps may be broken up by gentle trituration.
    IMPORTANT: Do not vortex the cells.
11. Count the number of cells using a hemocytometer.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of the Cells

N2A Psen1/Psen2 cells may be frozen in N2A Psen1/2 Expansion Medium supplemented with 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

References

1. Holtzman DM, Morris JC. Goate AM. *Sci Transl Med* 2011; 3(77): 77sr1.
2. Pimenova AA, Goate AM. *Neurobio Dis* 2020; 138:104785.
3. Lee E-S, Jeong S-J, Kim Y-H, Jeon C-J. *Acta Histochem Cytochem* 2015; 48(6): 205-214.
4. Olmsted JB, Carlson K, Klebe R, Ruddle F, Rosenbaum J. *Proc Natl Acad Sci USA* 1970; 65(1): 129-136.

ACADEMIC USE AGREEMENT

(subject to local law)

THIS PRODUCT MAY ONLY BE USED BY INDIVIDUALS EMPLOYED BY AN ACADEMIC INSTITUTION AND IS INTENDED SOLELY TO BE USED FOR ACADEMIC RESEARCH, WHICH IS FURTHER DEFINED BELOW. BY OPENING THIS PRODUCT, YOU ("PURCHASER") HEREBY REPRESENT THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF AND/OR YOUR EMPLOYER INSTITUTION, AS APPLICABLE, AND CONSENT TO BE LEGALLY BOUND BY THE TERMS OF THIS ACADEMIC USE AGREEMENT. IF YOU DO NOT AGREE TO COMPLY WITH THESE TERMS, YOU MAY NOT OPEN OR USE THE PRODUCT AND YOU MUST CALL MILLIPORESIGMA ("SELLER") CUSTOMER SERVICE (1-800-645-5476) TO ARRANGE TO RETURN THE PRODUCT FOR A REFUND.

"Product" means N2A Psen1/Psen2 Cell Line (SCC297).

"Academic Research" means any internal *in vitro* research use by individuals employed by an academic institution. Academic Research specifically excludes the following uses of whatever kind or nature:

- Re-engineering or copying the Product
- Making derivatives, modifications, or functional equivalents of the Product
- Obtaining patents or other intellectual property rights claiming use of the Product
- Using the Product in the development, testing, or manufacture of a Commercial Product
- Using the Product as a component of a Commercial Product
- Reselling or licensing the Product
- Using the Product in clinical or therapeutic applications including producing materials for clinical trials
- Administering the Product to humans
- Using the Product in collaboration with a commercial or non-academic entity

"Commercial Product" means *any* product intended for: (i) current or future sale; (ii) use in a fee-for-service; or (iii) any diagnostic, clinical, or therapeutic use.

Access to the Product is limited solely to those officers, employees, and students of PURCHASER's academic institution who need access to the Product to perform Academic Research. PURCHASER shall comply with all applicable laws in its use and handling of the Product and shall keep it under reasonably safe and secure conditions to prevent unauthorized use or access.

These use restrictions will remain in effect for as long as PURCHASER possesses the Product.

COMMERCIAL OR NON-ACADEMIC ENTITIES INTERESTED IN PURCHASING OR USING THE PRODUCT MUST CONTACT licensing@emdmillipore.com AND AGREE TO SEPARATE TERMS OF USE PRIOR TO USE OR PURCHASE.

GMO
This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient des organismes génétiquement modifiés.
Dit product bevat genetisch gewijzigde organismen.

The life science business of Merck KGaA, Darmstadt, Germany
operates as MilliporeSigma in the US and Canada.