Applied StemCell, Inc.
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Datasheet

Neurons
(iPSC from Blood Cells; Male)

Product Information

Catalog Number

ASE-9321 (Male)

Description

Applied StemCell's Neurons are cryo-preserved, pre-differentiated mixed population neuronal precursors derived from a footprint-free, karyotype normal human iPSC line. They yield high purity, functional neurons within 8 days when using Applied StemCell's well-optimized maturation medium and supplements. The derived-neurons have also been tested for neuronal activity using functional and screening assays. The neurons can be seeded on various culture vessel formats including 96-well plates on either glass or plastic surfaces and cultured as adherent cells. Shortly after seeding, the cells proliferate slightly for up to 3 days and show extensive neurite outgrowth and proper neuronal morphology. In general, on Day 8 post-seeding, the cell population will contain >90% neurons and < 5% Glial Fibrillary Acidic Protein (GFAP) positive astrocytes.

Characterization for Neurons derived from Applied StemCell NSCs

The neuronal maturation can be assessed by their morphology and by immunostaining. Immunostaining on day 10 post-seeding shows ≥98% of total cells expressed Tuj-1 marker (neuronal class β-III tubulin, green) and ≥40% expressed marker of GABAergic neurons GABA (red). Total count of nuclei (blue) is used as the total number of cells.

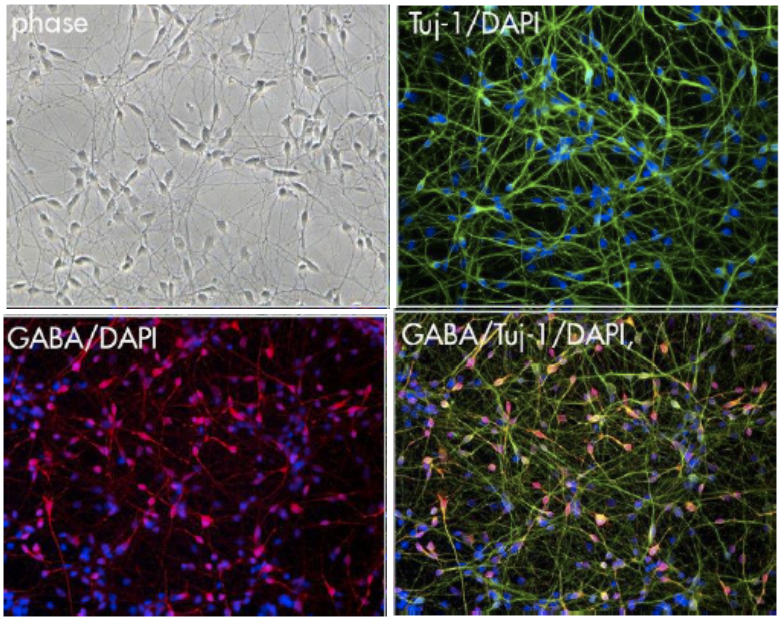

Figure 1. Immunocytochemical analysis of Applied StemCell Neurons. Green: neuronal marker Tuj-1 (neuronal type β III tubulin); Red: Gabaergic neurons; Blue: DAPI.

Quality Control

Neuron purity: ≥90% Tuj-1 positive; ≤5% GFAP positive
Recovery of frozen cells: ≥80% viability

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Amount	≥1.0x10^6 viable cells/vial
Shipping	Dry ice
Storage and Stability	Store in liquid nitrogen freezer immediately upon receipt. This product is stable for at least 6 months from the date of receiving when stored as directed.
Safety Precaution	**PLEASE READ BEFORE HANDLING ANY FROZEN VIALS**. Please wear the appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.
Restricted Use	This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.
Warranty	Performance of Applied StemCell's neurons has been extensively tested with other components. Applied StemCell will not hold responsibility if media other than the recommended maturation media is used.

Additional Reagents Required for neuron maturation

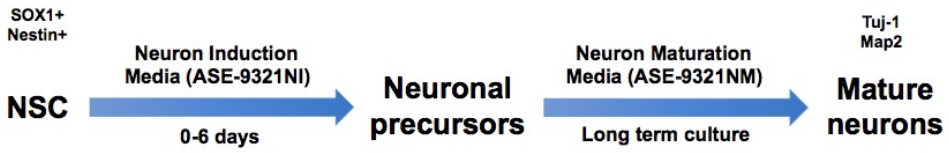
- Poly-L-ornithine hydrobromide, Sigma-Aldrich, Cat# P3655
- Primary antibodies:
  - Mouse anti-β III-tubulin isotype III clone SDL.3D10, Sigma, Cat# T8660
  - Rabbit anti-GFAP, Dako, Cat# M0761

Equipment required for neuron differentiation

Equipment	Specifications
Vertical laminar flow hood	
Incubator	Maintain 37°C and 95% humidity in an atmosphere of 5% CO2
Low speed centrifuge	(e.g. Thermo Centra CLS)
Pipette-aid	
Serological pipettes	
Hemacytometer or Automated Cell Counter	(e.g. BioRad TC 10)
Inverted microscope	(e.g. Nikon TS100)
Appropriate culture-ware	Tissue-treated plates (e.g. 430167, 430145, 430196, corning)

Protocol

Process of neuronal differentiation using Neuron Induction (ASE-9321NI) and Maturation Media (ASE-9321NM)



1. Handling Upon Receiving

Neuronal precursor cells are shipped on dry ice. Upon receiving the product, check the integration of the packages and the presence of dry ice (contact Applied StemCell if the integrity of the package has been compromised, e.g. no dry ice in the package)

Cells can be plated immediately after arrival or transferred into liquid nitrogen. Do not remove the vials from dry ice during transportation to storage units. Immediately transfer components (especially the cryopreserved precursor cells) to storage units, avoiding exposure to room temperature where required.

2. Coating Cell Culture Vessels with Poly-L-ornithine/Laminin

Cell culture vessels should be coated one day before or on the day of plating the cells.

2.1 Prepare a stock solution of poly-L-ornithine (10mg/mL) by dissolving the powder in sterile cell culture grade water. The stock solution should be aliquoted as desired and stored at -20°C.
2.2 Thaw a stock solution of poly-L-ornithine on ice.
2.3 Prepare working solution of poly-L-ornithine in sterile cell culture grade water (final concentration 20 µg/mL). To make sufficient volume for the experiment, please refer to Table 1.
2.4 Add poly-L-ornithine solution into desired cell culture vessel to cover growth surface entirely (see Table 1).
2.5 Distribute the solution evenly and incubate vessels in a cell culture incubator for 2 hours (37°C/ 5% CO_2/ humidity control).
2.6 In the meantime, thaw a stock solution of laminin (1 mg/mL) on ice.
2.7 Prepare a working solution of laminin in sterile cell culture grade water (f.c. 10 µg/mL). To make sufficient volume for the experiment, please refer to Table 1.
2.8 Rinse vessels twice with cell culture grade water. Pipette water gently toward the corner of the vessel to avoid mechanical removal of poly-L-ornithine coating.
2.9 Aspirate water from the vessels and add laminin solution to cover growth surface entirely. Incubate in the cell culture incubator (37°C/ 5% CO_2/ humidity control) for 2 hours.
2.10 We recommend that freshly coated vessels be used. However, if not used immediately, store coated vessels at 4°C in laminin solution (up to 4 days).
2.11 Pre-warm vessels at 37°C before use.
2.12 Aspirate laminin just before seeding cells. Do not let the surface dry. There is no need to wash vessels after removal of laminin.

Table 1. Recommended volumes of coating reagents for various vessels.

Vessel	Approx. Surface Area	poly-L-ornithine	Laminin
96 well plate	0.33 cm^2/well	50 µL/well	50 µL/well
4 or 24 well plate	2 cm^2/well	250 µL/well	250 µL/well
35 mm dish	10 cm^2	1.5 mL	1.5 mL
60 mm dish	20 cm^2	2.5 mL	2.5 mL

3. Maturation of Neuronal Precursors into Functional Neurons

During the neuron maturation phase, cell divisions will gradually decrease and neural precursors will elongate significantly, generate neuronal processes and mature.

All operations should be performed in accordance with aseptic cell culture standards. All media and vessels used for cell culture must be pre-warmed to 37°C prior to use.

3.1 Preparation of Complete Neuronal Maturation Media

Thaw media components overnight at 2°- 8°C. Complete medium shall be stored at 2°- 8°C and used within one week. Pre-warm an aliquot of complete medium at 37°C before use.

Table 2. Formulation of Complete Neuronal Maturation Medium (100 mL)

Component	Storage	Volume Provided	Formulation Per 50 mL	Optional one time re-freezing
Neuronal Maturation Basal Medium	Long term: -20°C Short term: 2-8°C (3 weeks)	1x100 mL	50 mL	yes
Neuronal Maturation Supplement A	-20°C	1x100 µL	50 µL	yes

3.2 Thawing and Culturing Neuronal Precursors

All steps described here must be performed in accordance with aseptic cell culture standards. All media and vessels used for cell culture must be pre-warmed to 37°C prior to use. For the entire process of neuronal maturation, one type of complete medium is required: Neuronal Maturation Basal Medium + Neuronal Maturation Supplement A (ASE-9321NM).

3.2.1 One day before thawing precursor cells, place the 50 mL Neuronal Maturation Medium bottle in 2°- 8°C fridge overnight.

Note: *Once thawed, Neuronal Medium can be stored at 2°- 8°C for up to 3 weeks.*

3.2.2 On the day of thawing precursor cells, transfer a 25 mL aliquot of Neuronal Medium into a 50 mL conical tube and add 25 µL of Neuronal Maturation Supplement A from vial 1 (pre-thawed on ice) to make the complete medium.

3.2.3 Transfer a 5 mL aliquot of the complete medium prepared in step 2 into a 15 mL conical tube and pre-warm at 37°C. This aliquot will be used for recovery of the precursor cells from frozen stock.

3.2.4 Prepare another aliquot of complete medium in accordance with volumes required for cell culture vessels utilized (see Table 3). Only take enough medium to be utilized for cell culture that day

and pre-warm at 37°C. Place the rest of the complete medium back into a 2°- 8°C fridge.

Table 3. Recommended volumes of medium in various vessels

Vessel Type	Medium Volume
96 well plate	100 µL/well
4 or 24 well plate	500 µL/well
35 mm dish	2 mL
60 mm dish	5 mL

3.2.5 Shortly before thawing the cells, place pre-warmed medium and vessels in the biosafety cabinet.
3.2.6 To thaw cryopreserved precursor cells, remove one vial from storage unit and place immediately onto dry ice (the vial must be buried in the dry ice).
3.2.7 Bring the dry ice container with the vial to a 37°C water bath and immerse the vial in the bath (up to ⅔ of the vial) and thaw cells until only a small piece of ice is still visible (approximately 1 minute).

Note: *Do not shake the vial during thawing.*

3.2.8 Immediately bring the vial to the biological cabinet, spraying it thoroughly with 70% ethanol and wiping with an autoclaved paper towel.
3.2.9 Remove cells from the vial using a p1000 micropipette (or serological pipette) and transfer drop-wise while swirling into the 15 mL conical tube containing 5 mL of pre-warmed complete medium (step 3). Wash the vial with 1 mL of medium from the 15 mL conical tube and transfer it back to the tube.

Note: *Do not mix cells up and down and avoid generating bubbles.*

3.2.10 Centrifuge cells at 400 x g for 5 minutes at room temperature.
3.2.11 Aspirate the medium very carefully using a vacuum (or pipette if preferred), leaving only a drop of liquid in the tube. Take extra care not to remove or disturb the cell pellet during aspiration of medium.
3.2.12 Using a p1000 micropipette, add 1 mL of complete medium (step 4) into the tube and gently re-suspend cells by pipetting up and down 4-6 times.
3.2.13 Remove a 10 µL aliquot of cell suspension and mix it with 10 µL of Trypan blue solution.
3.2.14 Count the cells.
3.2.15 Take the appropriate volume of pre-warmed complete Neuronal Medium and add to the tube with the cells.
3.2.16 Aspirate laminin solution from pre-warmed cell culture vessel and seed neurons at a density ranging from $4x10^4$ to $8x10^4$ live cells/cm^2. See Table 4 for your convenience.

Table 4. Recommended seeding densities for Applied StemCell neurons in various types of cell culture vessels. Range: low to high.

Vessel	Surface/Well	Medium Volume	Density (Cells)
96-well plate	0.33 cm^2	100 µL/well	$1.3 \times 10^4 – 2.6 \times 10^4$
4-well plate	2 cm^2	500 µL/well	$8 \times 10^4 – 1.6 \times 10^5$
35 mm dish	10 cm^2	2 mL	$4 \times 10^5 – 8 \times 10^5$
60 mm dish	20 cm^2	5 mL	$8 \times 10^5 – 1.6 \times 10^6$

3.2.17 Distribute cells evenly and place in vessels in the cell culture incubator (37°C/ 5% CO_2/humidity

control). This is **Day 0**; Medium should be changed ever other day.

3.2.18 Monitor the cells' survival and attachment the following day (**Day 1**).

3.2.19 Change complete Neuronal Maturation Medium on **Day 2**. Medium change should be done slowly (drop wise), pointing the pipette tip toward the wall of cell culture vessel.

3.2.20 Change medium every other day. Continue maturation of neurons for 8 days.

Note: *Cells can be differentiated for up to 5 weeks post-seeding. However, prolonged culture will have increased population of GFAP positive astrocytes.*