Applied StemCell, Inc.
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Datasheet

Neurons Starter Kit (iPSC from Blood Cells; Male)

Product Information

Catalog Number

ASE-9321K

Description

Applied StemCell's Neurons Starter Kit contains cryo-preserved, pre-differentiated mixed population neuron precursors derived from a footprint-free, karyotype normal human iPSC line, ASE-9109. It is designed for customers to generate mature neurons using Applied StemCell's optimized Neuron Maturation Medium and supplements. Mature neurons can be obtained within 8 days. 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.

Characterization of Mature Neurons

Maturation of neurons can be assessed by their morphology and by immunostaining of the Tuj-1 marker (neuronal class III-β tubulin). Percentage of neurons can be determined by a count of Tuj-1 positive neurons divided by the total number of cells (DAPI staining of nuclei).

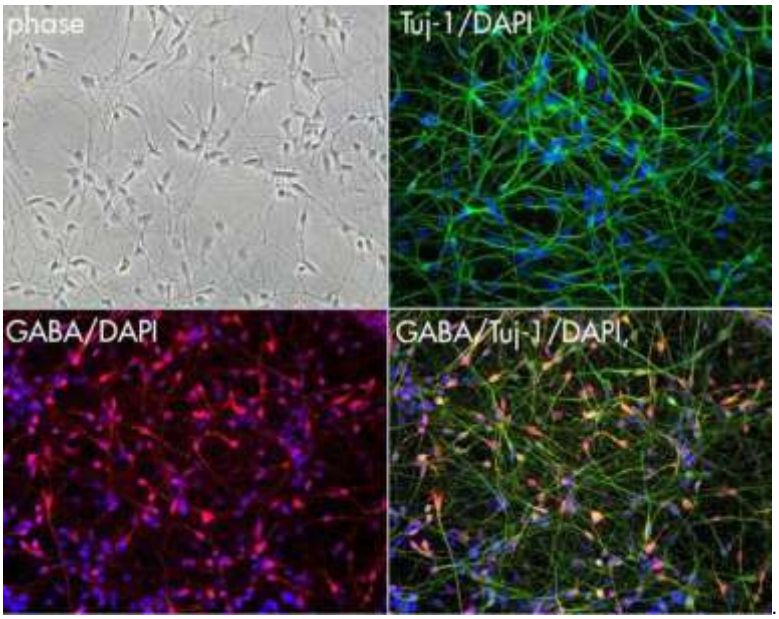

Figure 1. Example of mature neurons. Immunostaining on Day 10 post-deesing showing >90% of total cells expressed Tuj-1 marker (neuronal class β-III tubulin; green).

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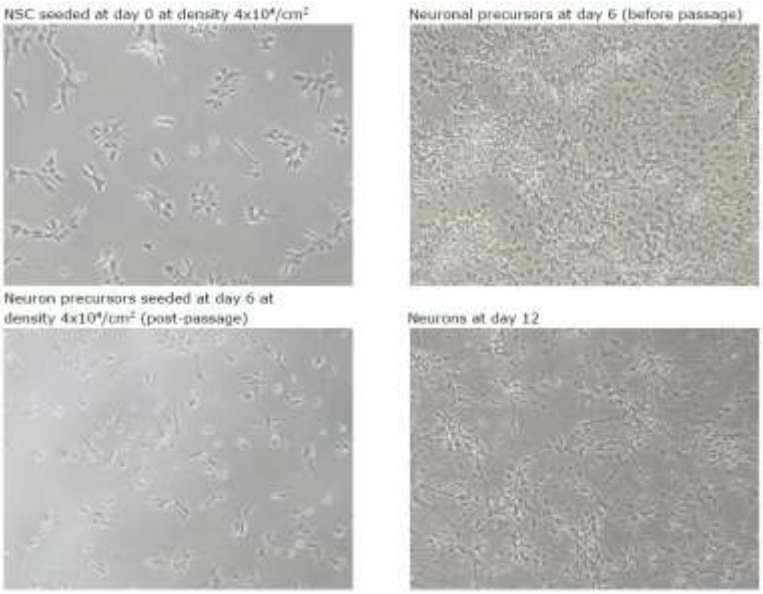


Figure 2. Example of NSC, neuronal precursors and neuron densities at different stages of differentiation using Neuronal Induction and Neuronal Maturation Media.

Shipping Dry ice

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 neuron precursors has been extensively tested with other components of the Neuron Starter Kit. Applied StemCell will not hold responsibility if components other than those provided with Neurons Starter Kit are used to culture neuron precursors.

Media and Material

Neurons Starter Kit (ASE-9321K)

Part #	Catalog #	Component	Amount	Storage	Shelf Life
ASE-9321K-c	ASE-9321	Neuron Precursors	1 vial ≥1 x 10^6 viable cells	Liquid Nitrogen	12 months
ASE-9321K-a	ASE-9321NM-a	Neuron Maturation Medium	1 x 100 mL	-20°C	18 months
ASE-9321K-b	ASE-9321NM-b	Neuron Maturation Supplement A	1 x 100 µL vial	-20°C	18 months

Additional Reagents Required

- Recommended:

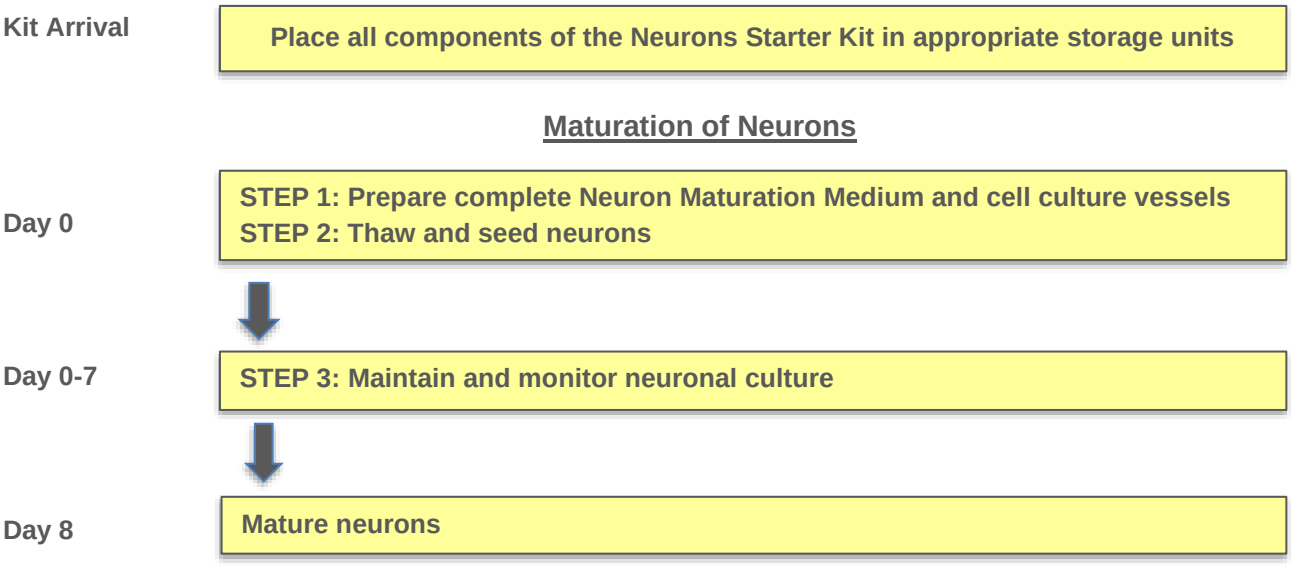
- Poly-L-ornithine hydrobromide, Sigma, Cat# P3655
- Laminin, ThermoFisher, Cat# 23017015

- Primary antibodies:
  - Mouse anti-β III-tubulin isotype III clone SDL.3D10, Sigma, Cat# T8660

Protocol

Notes:
- *We do not recommend re-freezing supplements and medium provided with the Neurons Starter Kit.*
- *We do not recommend cryopreserving Neurons Starter Kit cultured neurons.*

Simplified diagram showing key steps in the process of culturing Applied StemCell neurons



1. Handling Upon Receiving

Neurons Starter Kit is shipped on dry ice. A vial containing cryopreserved neuronal precursors is packed in a small transparent bag which is buried in dry ice. Upon receiving the product, check integration of the package 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 of the Neurons Starter Kit (especially the cryopreserved neuronal precursors) to storage units, avoiding exposure to room temperature.

After arrival, properly store the kit's components as follows:

Component	Storage
Neuronal precursors	Liquid Nitrogen
Neuron Maturation Medium	-20°C
Supplement A	-20°C

2. Procedure

This procedure has been extensively tested with Applied StemCell neurons and Applied StemCell neuron maturation medium. The user should strictly follow provided protocol. The user assumes all responsibility for the failure of the experiment should there be any deviation from this procedure. Please read and understand the entire protocol before proceeding with the maturation procedure.

2.1 Coating Cell Culture Vessels with Poly-L-ornithine and Laminin

Cell culture vessels should be coated one day before or on the day of plating the cells. Please read producer's manual for handling of Poly-L-ornithine and Laminin.

2.1.1 Prepare stock solution of poly-L-ornithine (10 mg/mL) by dissolving the powder in sterile cell culture grade water. The stock solution should be stored at -20°C.
2.1.2 Thaw stock solution of laminin (1 mg/mL, ThermoFisher) on ice.
2.1.3 Prepare a working solution of poly-L-ornithine in sterile cell culture grade water at a final concentration of 20 µg/mL.
2.1.4 Add poly-L-ornithine solution into desired cell culture vessel to cover the vessel's bottom entirely (see Table 1).
2.1.5 Distribute the solution evenly and incubate vessels in the cell culture incubator for 2 hr (37°C/ 5% CO_2/ humidity control).
2.1.6 Rinse vessels two times with cell culture grade water. Pipette water gently toward the corner of the vessel to avoid mechanical removal of poly-L-ornithine coating.
2.1.7 Prepare working solution of laminin in sterile cell culture grade water at a final concentration of 10 µg/mL.
2.1.8 Aspirate water from the vessels and add laminin solution to cover well the vessel's bottom. Incubate in the cell culture incubator (37°C/ 5%CO_2/ humidity control) for 2 hours.
2.1.9 We advise freshly coated vessels be used. However, if not used immediately, store coated vessels at 4°C in laminin solution (up to 4 days).
2.1.10 Pre-warm vessels at 37°C before use.
2.1.11 Aspirate laminin just before seeding DOPA precursors. Do not let the surface dry.

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

Vessel Type	P-L-ornithine	Laminin
96 well plate	50 µL/well	50 µL/well
4 or 24 well plate	250 µL/well	250 µL/well
35 mm dish	1.5 mL	1.5 mL
60 mm dish	2.5 mL	2.5 mL

2.2 Thawing and Culturing Cryopreserved Neurons

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 neuron maturation, one type of complete medium is required: Neuron Maturation Medium + Supplement A.

2.2.1 One day before thawing precursor cells, place the 100 mL Neuron Maturation Medium bottle in 2°-8°C fridge overnight.

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

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

2.2.3 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 precursor cells from frozen stock.

2.2.4 Prepare another aliquot of the complete medium in accordance with volumes required for cell culture vessels utilized (see Table 2). 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 to 2°- 8°C fridge.

Table 2. 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

2.2.5 Shortly before thawing the cells, place pre-warmed medium and vessels in a biosafety cabinet.

2.2.6 To thaw cryopreserved neurons, remove one vial from storage unit and place immediately onto dry ice (the vial must be buried in the dry ice).

2.2.7 Bring dry ice container with the vial to a 37°C water bath and immerse the vial in the bath (up to 2/3rd of the vial) and thaw cells until only a small piece of ice is still visible (approximately one minute).

Note: Do not shake the vial during thawing.

2.2.8 Immediately bring the vial to the biological cabinet, spraying it thoroughly with 70% ethanol and wiping with an autoclaved paper towel.

2.2.9 Remove cells from the vial using a p1000 micropipette (or serological pipette) and transfer drop-wise while swirling into 15 mL conical tube containing 5 mL of pre-warmed complete medium (step 3). Wash the vial with 1 mL medium from the 15 mL conical tube and transfer it back to the tube.

Note: Do not mix cells up and down and avoid generation of bubbles.

2.2.10 Centrifuge cells at 300 x g for 5 minutes at room temperature.

2.2.11 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.

2.2.12 Using a p1000 micropipette, add 1 mL of the complete medium (step 4) into the tube and gently re-suspend cells by pipetting up and down 4-6 times.

2.2.13 Remove a 10 µL aliquot of cell suspension and mix it with 10 µL of Trypan blue solution.

2.2.14 Count the cells.

2.2.15 Take the appropriate volume of pre-warmed complete Neuron Maturation Medium and add to the tube with the cells.

2.2.16 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 3 for your convenience.

Table 3. Recommended seeding densities for Applied StemCell neurons in various types of cell culture vessels. Range: from low to high.

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

2.2.17 Distribute cells evenly and place vessels in the cell culture incubator (37°C/ 5% CO_2/humidity control). This is **Day 0**; Medium should be changed ever other day.

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

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

2.2.20 Change medium every other day. Continue maturation of neurons for 8 days.
Note: Cells can be differentiated for up to 5 weeks. However, prolonged culture will have increased population of GFAP positive astrocytes.