Applied StemCell, Inc.
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Datasheet

Neuron Maturation Media

Product Information

Catalog Number ASE-9321NM

Description Neuron Maturation Media is serum-free media produced using Applied StemCell's proprietary formulations to allow researchers to differentiate neural stem cells (NSC) into functional neurons. Applied StemCell's induction and maturation media have been extensively tested and optimized using NSC and neurons derived from a variety of human pluripotent cells (hESC and hiPSC). The neuronal differentiation process is divided into two stages. In the first stage, the Neuron Induction Medium is used to differentiate NSC into neuronal precursors. In the second stage, the Neuron Maturation Medium is used to further differentiate neuronal precursors into mature, functional neurons and to maintain mature neurons in long-term culture (up to 40 days) (See Figure 1). Neuron Induction Medium can be purchased separately (ASE-9321NI) or as part of the Neuron Starter Kit (ASE-9321K/ ASE-9321KF).

Figure 1. The process of neuronal differentiation using Neuronal Induction and Maturation Media.

Quantity

Neuron Maturation Basal Medium	100 mL
Neuron Maturation Supplement A	100 µL

Shipping Dry ice

Storage and Stability Store at -20°C. 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.

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Warranty

Performance of Applied StemCell's Neuron Maturation Media has been extensively tested with other components. Applied StemCell will not hold responsibility if components other than the recommended components are used.

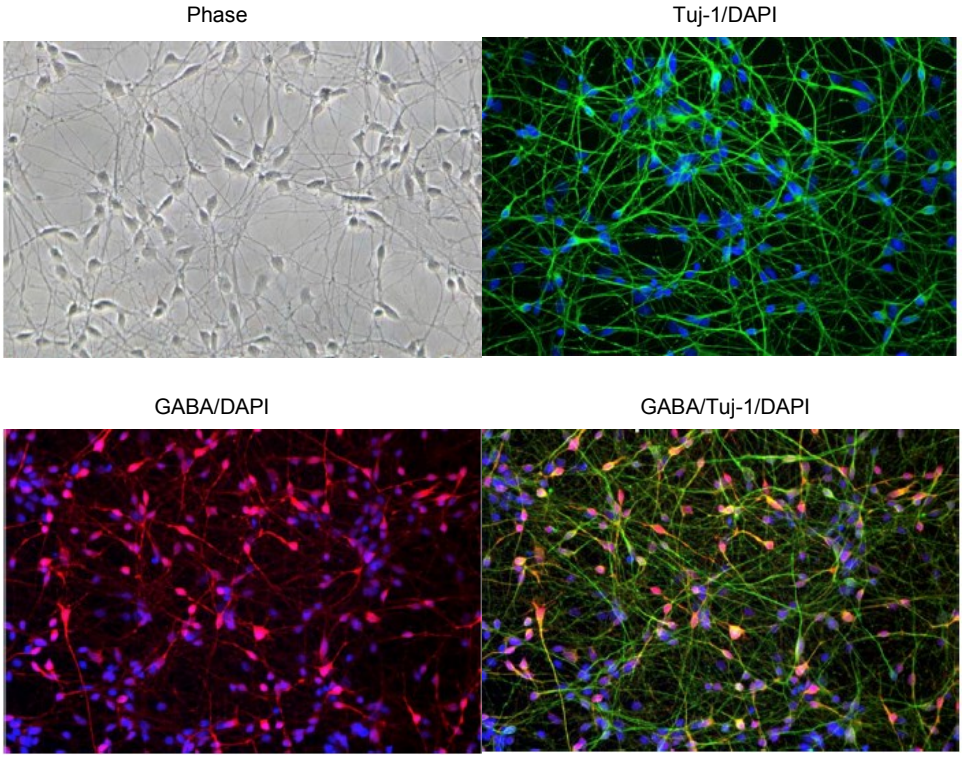

Figure 3. Neuron morphology and immunostaining data at 10 days post-thaw. Tuj-1 (Neuronal Class III β-Tubulin) – green, GABA (GAMMA – AMINOBUYRIC ACID) - red

Media and Material

Components included with Neuron Maturation Media (ASE-9321NM):

Cat. Number	Component	Amount	Storage
ASE-9321NM-A	Neuron Maturation Basal Medium	100 mL	Long-term: -20°C Short-term: 2-8°C
ASE-9321NM-B	Neuron Maturation Supplement A	100 µL	-20°C

Additional Reagents Required but not Provided

- Poly-L-ornithine hydrobromide, Sigma-Aldrich, Cat# P3655
- Laminin, Life Technologies, Cat# 23017-015
- Cell culture grade water, Corning Cellgro, Cat# 25-055-CVC
- Accutase (cell dissociation reagent), Life Technologies, Cat# A11105-01
- DMEM, Life Technologies, Cat# 12491-015

Protocol

1. Handling Upon Receiving

Components of Applied StemCell's Maturation Media are shipped on dry ice. Upon receiving the product, check

the integrity of the packages and the presence of dry ice (contact Applied StemCell if the integrity of a package has been compromised, e.g. no dry ice in the package). Store components as specified.

2. Preparation of Culture Vessels and Media

The handling procedures described below have been extensively tested for Applied StemCell's neurons (ASE-9321, ASE-9321F) using specified substrate coating and Applied StemCell's optimized maturation media. The user should follow these procedures closely. The user assumes all responsibility for the failure of the experiment should there be any deviation from these procedures.

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 a stock solution of poly-L-ornithine on ice.
2.1.3 Prepare working solution of poly-L-ornithine in sterile cell culture grade water (f.c. 20 µg/mL). To make sufficient volume for this experiment, please refer to Table 1.
2.1.4 Add poly-L-ornithine solution into desired cell culture vessel to cover growth surface 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 In the meantime, thaw a stock solution of Laminin (1 mg/mL) on ice.
2.1.7 Prepare working solution of Laminin in sterile cell culture grade water (f.c. 20 µg/mL). To make sufficient volume for the experiment, please refer to Table 1.
2.1.8 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.
2.1.9 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.
2.1.10 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).
2.1.11 Pre-warm vessels at 37°C before use.
2.1.12 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 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

3. Neuronal Maturation

The neuronal differentiation process is comprised of two phases. I) During the induction phase, differentiation of NSC into neuronal precursors will be initiated, which will be hallmarked by morphological changes in NSC (cell polarization and elongation), however the cells will still proliferate. II) During the maturation phase, cell divisions will gradually decrease and neural precursors will elongate significantly, generate neuronal processes and mature.

All steps described below were optimized using neurons and precursors produced by Applied StemCell from parental control iPSC lines (ASE-9109 and ASE-9110). If neurons from other sources are used, the protocols might need to be further optimized for these cells. 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.

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 3 weeks. Pre-warm an aliquot of complete medium at 37°C before use.

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

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	1 x 100 mL	50 mL	yes
Neuronal Maturation Supplement A	-20°C	2 x 50 µL	50 µL	yes

3.1 Aspirate Laminin solution from pre-warmed cell culture vessel and seed neuronal precursors at a density ranging from 4 x 10^4 to 6 x 10^4 live cells/cm^2 in Neuron Maturation Medium. Suggested volumes are listed in Table 3.

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 Distribute cells evenly and place vessels in the cell culture incubator (37°C/ 5%CO_2/ humidity control).
3.3 Change medium every alternate day. To avoid cell peeling, media change should be done slowly (drop-wise) pointing the pipette tip toward the wall of cell culture vessel.
3.4 Continue maturation of neurons for a minimum of 8 days. Neurons can be cultured for up to 5 weeks if prolonged maturation time is required.