BrainPhys™ Neuronal Medium

Serum-free neurophysiological basal medium for improved neuronal function

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Product Description

BrainPhys™ Neuronal Medium is a defined and serum-free neuronal basal medium. BrainPhys™ may be used to culture primary tissue-derived neurons or human pluripotent stem cell (hPSC)-derived neurons. Based on the formulation published by Cedric Bardy and Fred H. Gage[1], BrainPhys™ is more representative of the central nervous system (CNS) extracellular environment and increases the proportion of synaptically active neurons.

Ordering Information

PRODUCT NAME	CATALOG #	SIZE	KIT COMPONENTS
BrainPhys™ Neuronal Medium	05790	500 mL	Not applicable.
BrainPhys™ Without Phenol Red	05791	500 mL	Not applicable.
BrainPhys™ Neuronal Medium and SM1 Kit	05792	1 Kit	• BrainPhys™ Neuronal Medium • NeuroCult™ SM1 Neuronal Supplement
BrainPhys™ Neuronal Medium and N2-A & SM1 Kit	05793	1 Kit	• BrainPhys™ Neuronal Medium • NeuroCult™ SM1 Neuronal Supplement • N2 Supplement-A

Storage and Stability

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
BrainPhys™ Neuronal Medium	05790	500 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
BrainPhys™ Without Phenol Red	05791	500 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
NeuroCult™ SM1 Neuronal Supplement*	05711	10 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
N2 Supplement-A**	07152	5 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

*Please refer to the Safety Data Sheet (SDS) for hazard information. Lot-to-lot color variations include light to dark yellow or orange. This will not affect performance.
**This product contains components derived from human plasma. Donors have been tested and found negative for hepatitis B surface antigen (HBsAg) and HIV-1 antibodies and/or HIV-1 antigen. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Directions for Use

Protocols are provided below for A: Culture of Primary Tissue-Derived Neurons and B: Neuronal Differentiation of hPSC-Derived Neural Progenitor Cells. Select the appropriate protocol for your cell type.

A. CULTURE OF PRIMARY TISSUE-DERIVED NEURONS

Please read the entire protocol before proceeding.

Preparation of Poly-D-Lysine (PDL)-Coated Culture Surface

NOTE: Cells can be cultured on tissue culture-treated plasticware or on glass coverslips.

1. If culturing cells on coverslips, use sterile forceps to place a sterile round glass coverslip at the bottom of an individual well of a 24-well plate.
2. Dissolve 5 mg of PDL (Sigma Catalog #P7280) in 50 mL of sterile water to give a final concentration of 100 µg/mL.
   NOTE: Aliquot solution in polypropylene vials and store at 2 - 8°C for up to 1 month.
3. Dilute the 100 µg/mL PDL stock solution with sterile water to a final concentration of 10 µg/mL.
4. Dispense 0.5 mL of 10 µg/mL PDL solution into each well of a 24-well plate that will be used for culturing.
   NOTE: If using coverslips, ensure that the coverslips are completely submerged in the PDL solution, as the coverslips tend to float. If this happens, use a sterile plastic disposable pipette tip to push the coverslip to the bottom of the well.
5. Incubate at room temperature (15 - 25°C) for 2 hours or overnight at 2 - 8°C.
   NOTE: If not used the same day, wrap the plate with Parafilm® and store at 2 - 8°C for up to 2 weeks.
6. At the end of the incubation, wash each well 2 times with 1 mL of sterile PBS. When ready to plate the cells, remove the PBS. Do not allow the coated coverslips or wells to completely dry.
   NOTE: DMEM/F-12 can also be used for washes.

Preparation of Media

NOTE: BrainPhys™ Without Phenol Red may be used in place of BrainPhys™ Neuronal Medium in the protocols below.

Complete Plating Medium

Use sterile technique to prepare Complete Plating Medium (NeuroCult™ Neuronal Basal Medium [Catalog #05710] or Neurobasal® Medium [Thermo Fisher Catalog #21103-049] + NeuroCult™ SM1 Neuronal Supplement [Catalog #05711] + L-glutamine + L-glutamic acid). The following example is for preparing 10 mL of medium. If preparing other volumes, adjust accordingly.

1. Thaw one bottle of NeuroCult™ SM1 at room temperature (15 - 25°C) for 1 hour.
   NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the expiry date (EXP) as indicated on the label.
2. Add 0.2 mL of NeuroCult™ SM1 to 9.8 mL of NeuroCult™ Neuronal Basal Medium or Neurobasal® Medium (1 in 50 dilution).
3. Add the following supplements and mix thoroughly:
   - 25 µL of 200 mM L-Glutamine (Catalog #07100)
   - 18.5 µL of 2 mg/mL L-Glutamic Acid

   NOTE: If not used immediately, store Complete Plating Medium at 2 - 8°C for up to 1 month.

Complete Maturation Medium

Use sterile technique to prepare Complete Maturation Medium (BrainPhys™ Neuronal Medium + NeuroCult™ SM1 Neuronal Supplement [Catalog #05711]). The following example is for preparing 10 mL of medium. If preparing other volumes, adjust accordingly.

1. Thaw one bottle of NeuroCult™ SM1 at room temperature (15 - 25°C) for 1 hour.
   NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the expiry date (EXP) as indicated on the label.
2. Add 0.2 mL of NeuroCult™ SM1 to 9.8 mL of BrainPhys™ Neuronal Medium (1 in 50 dilution). Mix thoroughly.
   NOTE: If not used immediately, store Complete Maturation Medium at 2 - 8°C for up to 1 month.

Culture of Primary Tissue-Derived Neurons

Indicated volumes are for a single well of a 24-well plate. If using other cultureware, adjust volumes accordingly.

1. Resuspend cells with Complete Plating Medium (see Preparation of Media) to obtain a final concentration of 3.9 x 10^6 cells/mL.
2. The cell density may be adjusted for different applications, as follows:

   For immunocytochemistry applications, plate cells at 3.2 x 10^4 cells/cm^2; add 20 µL cell suspension to each 1.3 mL Complete Plating Medium.

   OR

   For electrophysiology applications, plate cells at 4.8 x 10^4 cells/cm^2; add 30 µL cell suspension to each 1.3 mL Complete Plating Medium.
3. Mix the cells gently and add 1 mL of the cell suspension to a PDL-coated well (or a well containing a PDL-coated coverslip) of a 24-well plate.
4. Day 0: Incubate cultures at 37°C and 5% CO_2.
5. Day 1: Observe the cells to determine whether the cultures are viable; cells should be attached and minimal cell debris should be visible.
6. Day 5: Remove half (~ 0.5 mL) of the plating medium from each well. Replenish with the same volume of fresh Complete Maturation Medium (see Preparation of Media).
7. For extended culture periods, perform a half-medium change as described in step 6 every 3 - 4 days for the remainder of the culture period. Neurons have been cultured for up to 21 days using this protocol.
8. Upon reaching the end of the desired culture period, cells can be processed for immunocytochemistry or other applications.

B. NEURONAL DIFFERENTIATION OF hPSC-DERIVED NEURAL PROGENITOR CELLS

Please read the entire protocol before proceeding.

Preparation of Poly-L-Ornithine (PLO)/Laminin-Coated Culture Surface

NOTE: Cells can be cultured on tissue culture-treated plasticware or on glass coverslips.

1. If culturing cells on coverslips, use sterile forceps to place a sterile round glass coverslip at the bottom of an individual well of a 24-well plate.
2. Dilute 0.1% PLO solution (Sigma Catalog #P4957) with phosphate-buffered saline (PBS) to give a final concentration of 15 µg/mL.
3. Dispense 0.5 mL of 15 µg/mL PLO solution into each well of a 24-well plate that will be used for culturing.

   NOTE: If using coverslips, ensure that the coverslips are completely submerged in the PLO solution, as the coverslips tend to float. If this happens, use a sterile plastic disposable pipette tip to push the coverslip to the bottom of the well.
4. Incubate at room temperature (15 - 25°C) for 2 hours or overnight at 2 - 8°C.
5. Dilute 1 mg/mL laminin stock solution (Sigma Catalog #L2020) with sterile PBS (or DMEM/F-12) to a final concentration of 10 µg/mL.
6. At the end of the incubation, wash each well 2 times with 1 mL of sterile PBS. Remove as much of the PBS as possible from the wells.
7. Dispense 0.5 mL of 10 µg/mL laminin into each well.
8. Incubate at room temperature (15 - 25°C) for 2 hours or overnight at 2 - 8°C.

   NOTE: If not used the same day, wrap the plate with Parafilm® and store at 2 - 8°C for up to 2 weeks.
9. At the end of the incubation, wash each well 2 times with 1 mL of sterile PBS. When ready to plate the cells, remove the PBS. Do not allow the coated coverslips or wells to completely dry.

   NOTE: DMEM/F-12 can also be used for washes.

Preparation of Complete Differentiation Medium

NOTE: BrainPhys™ Without Phenol Red may be used in place of BrainPhys™ Neuronal Medium in the protocols below.

Use sterile technique to prepare Complete Differentiation Medium (BrainPhys™ Neuronal Medium + supplements). The following example is for preparing 10 mL of medium. If preparing other volumes, adjust accordingly.

1. Add the following supplements to 10 mL of BrainPhys™ Neuronal Medium:
   - 200 µL NeuroCult™ SM1 Neuronal Supplement (Catalog #05711)
   - 100 µL N2 Supplement-A (Catalog #07152)
   - 2 µL of 100 µg/mL Recombinant Human Brain-Derived Neurotrophic Factor (BDNF, Catalog#78005; final concentration 20 ng/mL)
   - 2 µL of 100 µg/mL Recombinant Human Glial-Derived Neurotrophic Factor (GDNF, Catalog#78058; final concentration 20 ng/mL)
   - 50 µL of 100 mg/mL dibutyryl cAMP (final concentration 1 mM)
   - 7 µL of 50 µg/mL ascorbic acid (final concentration 200 nM)
2. Mix thoroughly.

   NOTE: If not used immediately, store Complete Differentiation Medium at 2 - 8°C for up to 2 weeks.

Neuronal Differentiation

BrainPhys™ is compatible with neural progenitor cells (NPCs) generated using several methods, including STEMdiff™ Neural Induction Medium (Catalog #05835; Document #28782), and is also compatible with cryopreserved neuronal precursor cells (Catalog #70905/70908; Document #DX20718).

Indicated volumes are for a single well of a 24-well plate. If using other cultureware, adjust volumes accordingly.

1. Seed cells onto PLO/laminin-coated dishes at a density of 1.5 x 10^4 - 6.0 x 10^4 cells/cm^2 in 0.5 mL of the medium in which the cells were maintained. Distribute cells evenly. Incubate at 37°C and 5% CO_2.

   NOTE: **The seeding density of cells should be optimized for the application and the cell line.** For long-term cultures (> 30 days of maturation) and for immunocytochemistry, seed cells at 1.5 x 10^4 - 3 x 10^4 cells/cm^2. For short-term cultures (< 30 days of maturation), seed cells at 4 x 10^4 - 6 x 10^4 cells/cm^2.
2. The next day, add 0.5 mL of Complete Differentiation Medium to the existing culture medium. Incubate at 37°C and 5% CO_2.
3. Perform a half-medium change every 2 - 3 days as follows:
   a. Remove half of the culture medium (~0.5 mL).
   b. Add 0.5 mL of fresh Complete Differentiation Medium.
4. Continue to incubate cells at 37°C and 5% CO_2 for 2 - 4 weeks until cells begin to differentiate and neuronal morphology becomes apparent.
5. Analyze for neuronal differentiation using markers such as beta-tubulin III (e.g. using Anti-Beta-Tubulin III Antibody, Clone TUJ1 [Catalog #60052]), MAP2, and synapsin1.

References

1. Bardy C et al. (2015) Neuronal medium that supports basic synaptic functions and activity of human neurons in vitro. Proc Natl Acad Sci. 112(20):E2725-34.