

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 Bardy et al., BrainPhys™ is more representative of the central nervous system (CNS) extracellular environment and increases the proportion of synaptically active neurons.

The formulation contains reduced levels of neuroactive compounds compared to standard media (KCl, CaCl₂, MgCl₂, Fe(NO₃)₃, CuSO₄, FeSO₄, glycine, L-alanine, L-aspartic acid, L-glutamic acid, and L-serine). The glucose levels are at similar physiological levels to human cerebral spinal fluid (2.5 mM), and further energetic substrates are provided by sodium pyruvate (0.5 mM). The buffering system is composed of HEPES (5 mM) and sodium bicarbonate (29 mM). Key metabolites are present, including methionine (< 0.2 mM), lysine (< 0.8 mM), arginine (< 0.5 mM), and folate (< 0.006 mM). To request custom BrainPhys™ formulations, contact us at info@stemcell.com.

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. |
| NeuroCult™ Neuronal Plating Medium | 05713 | 100 mL | Not applicable. |
| BrainPhys™ Neuronal Medium and SM1 Kit | 05792 | 1 Kit | <ul style="list-style-type: none">BrainPhys™ Neuronal MediumNeuroCult™ SM1 Neuronal Supplement |
| BrainPhys™ Neuronal Medium N2-A & SM1 Kit | 05793 | 1 Kit | <ul style="list-style-type: none">BrainPhys™ Neuronal MediumNeuroCult™ SM1 Neuronal SupplementN2 Supplement-A |
| BrainPhys™ Primary Neuron Kit | 05794 | 1 Kit | <ul style="list-style-type: none">BrainPhys™ Neuronal MediumNeuroCult™ SM1 Neuronal SupplementNeuroCult™ Neuronal Plating Medium |
| BrainPhys™ hPSC Neuron Kit | 05795 | 1 Kit | <ul style="list-style-type: none">BrainPhys™ Neuronal MediumNeuroCult™ SM1 Neuronal SupplementN2 Supplement-AHuman Recombinant BDNFHuman Recombinant GDNF |

Storage and Stability

| PRODUCT NAME | CATALOG # | SIZE | STORAGE | SHELF LIFE |
|--------------------------------|-----------|--------|-------------------|---|
| BrainPhys™ Neuronal Medium* | 05790 | 500 mL | Store at 2 - 8°C. | Stable for 2 years from date of manufacture (MFG) on label. |
| BrainPhys™ Without Phenol Red* | 05791 | 500 mL | Store at 2 - 8°C. | Stable for 2 years from date of manufacture (MFG) on label. |

*Protect from light.

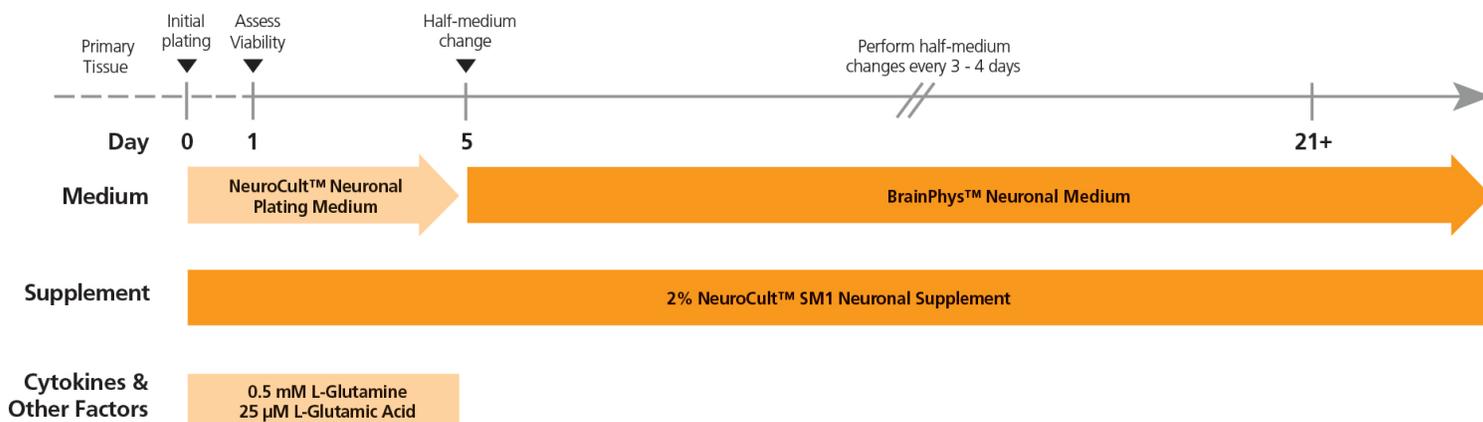
NOTE: These products may be shipped with ice packs or under ambient conditions. Store at 2 - 8°C upon receipt.

Directions for Use

Protocols are provided below for A: Culture of Primary Tissue-Derived Neurons, B: Neuronal Differentiation of hPSC-Derived Neural Progenitor Cells, and C: Glucose Supplementation for High-Density Neuronal Cultures and Neural Organoid Cultures. Select the appropriate protocol for your cell type.

A. CULTURE OF PRIMARY TISSUE-DERIVED NEURONS

Protocol Diagram



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.

- Dissolve 5 mg of PDL in 50 mL of sterile water to obtain a 100 μg/mL stock solution.
NOTE: Do not prepare the PDL solution with borate buffer. Increased cell clumping is observed when the PDL solution is prepared in borate buffer, and then cells are cultured in medium supplemented with NeuroCult™ SM1.
NOTE: If not used immediately, aliquot PDL stock solution into polypropylene vials and store at 2 - 8°C for up to 1 month.

- Coat culture wells or glass coverslips with PDL as described below:

Coating Culture Wells

- Dilute the 100 μg/mL PDL stock solution (prepared in step 1) with sterile water to 10 - 50 μg/mL.
- Dispense 0.5 mL of 10 - 50 μg/mL PDL into each well of a 24-well plate.
- Proceed to step 3.

Coating Glass Coverslips

- Use sterile forceps to place a sterile round glass coverslip in each well of a 24-well plate.
- Dilute the 100 μg/mL PDL stock solution (prepared in step 1) with sterile water to 40 μg/mL.
- Dispense 0.5 mL of 40 μg/mL PDL into each well of the plate prepared in step a.
NOTE: Ensure that the coverslips are completely submerged in the PDL solution, as they tend to float. If this happens, use a sterile plastic pipette tip to push the coverslip to the bottom of the well.
- Proceed to step 3.

- Incubate at room temperature (15 - 25°C) for 2 hours, or wrap the plate with Parafilm® and incubate overnight at 2 - 8°C.
- Wash each well with 2 x 1 mL of sterile phosphate-buffered saline (PBS), leaving PBS in the wells.

NOTE: DMEM/F-12 with 15 mM HEPES (Catalog #36254) can also be used for washes.

NOTE: If not used immediately, wrap the plate with Parafilm® and store at 2 - 8°C for up to 2 weeks.

- When ready to plate the cells, remove the PBS (or DMEM/F-12) from the wells. Do not allow the coated coverslips or wells to completely dry.

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 Plating Medium [Catalog #05713] + NeuroCult™ SM1 Neuronal Supplement [Catalog #05711] + L-Glutamine + L-glutamic acid). The following example is for preparing 10 mL of complete 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 as indicated on the label.
2. Add 0.2 mL of NeuroCult™ SM1 to 9.8 mL of NeuroCult™ Neuronal Plating 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 complete 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 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. Protect from light.

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 3.9×10^6 cells/mL.
2. The cell density may be adjusted for different applications, as follows:

For immunocytochemistry applications, plate cells at 3.2×10^4 cells/cm²; add 20 µL cell suspension to each 1.3 mL of Complete Plating Medium.

OR

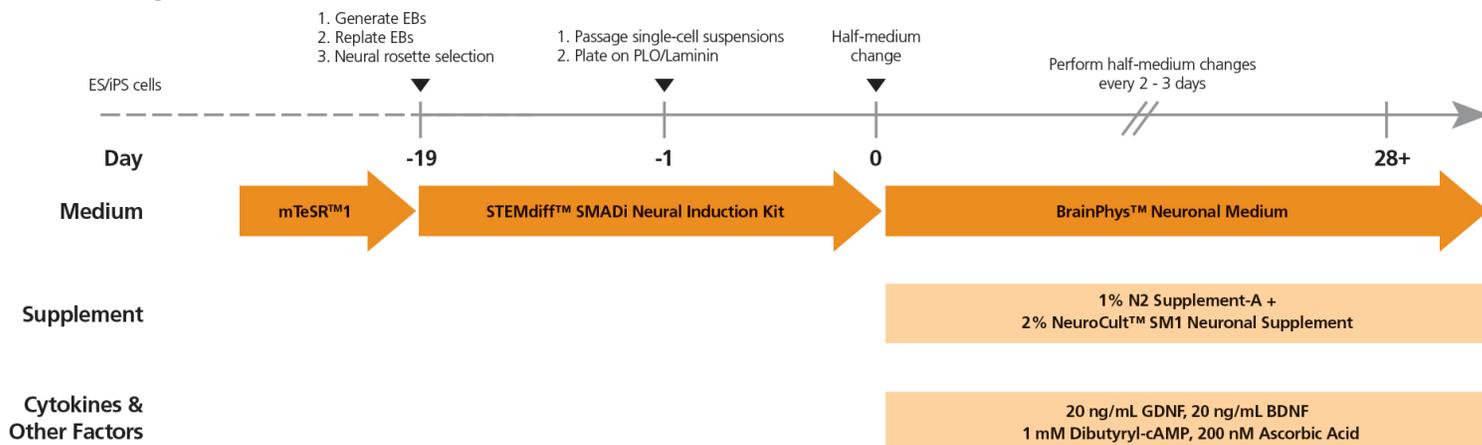
For electrophysiology applications, plate cells at 4.8×10^4 cells/cm²; add 30 µL cell suspension to each 1.3 mL of Complete Plating Medium.

3. Using a pipettor set at 1 mL, mix the cells gently. 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% CO₂.
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 of the plating medium (~ 0.5 mL) 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.

NOTE: BrainPhys™ Neuronal Medium is a versatile basal medium suitable for supporting the culture of primary tissue-derived neurons in various protocols. However, BrainPhys™ Neuronal Medium should not be used as a plating medium. To transition from traditional medium (e.g. DMEM/F-12), introduce BrainPhys™ Neuronal Medium on or after Day 5 by removing half of the traditional medium from each well and replacing it with an equal volume of BrainPhys™ Neuronal Medium, in combination with supplements and cytokines of choice. Continue performing half-medium changes every 3 - 4 days until the end of the culture period.

B. NEURONAL DIFFERENTIATION OF hPSC-DERIVED NEURAL PROGENITOR CELLS

Protocol Diagram



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.

- 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.
 - Dilute 0.01% PLO solution (Sigma Catalog #P4957) with phosphate-buffered saline (PBS) to give a final concentration of 15 µg/mL.
 - 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.
- Incubate at room temperature (15 - 25°C) for 2 hours, or wrap the plate with Parafilm® and incubate overnight at 2 - 8°C.
 - 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.
 - After incubation, wash each well with 2 x 1 mL of sterile PBS. Remove as much of the PBS as possible from the wells.
 - Dispense 0.5 mL of 10 µg/mL laminin into each well.
 - Incubate at room temperature for 2 hours, or wrap the plate with Parafilm® and incubate overnight at 2 - 8°C.
 - Wash each well with 2 x 1 mL of sterile PBS, leaving PBS in the wells.

NOTE: DMEM/F-12 with 15 mM HEPES can also be used for washes.

NOTE: If not used immediately, wrap the plate with Parafilm® and store at 2 - 8°C for up to 2 weeks.

- When ready to plate the cells, remove the PBS (or DMEM/F-12) from the wells. Do not allow the coated coverslips or wells to completely dry.

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 complete medium. If preparing other volumes, adjust accordingly.

- 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 Human Recombinant BDNF (Catalog #78005; final concentration 20 ng/mL)
 - 2 µL of 100 µg/mL Human Recombinant GDNF (Catalog #78058; final concentration 20 ng/mL)
 - 50 µL of 100 mg/mL Dibutyryl-cAMP (Catalog #73882; final concentration 1 mM)
 - 7 µL of 50 µg/mL Ascorbic Acid (Catalog #72132; final concentration 200 nM)
- Mix thoroughly.

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

Neuronal Differentiation

BrainPhys™ Neuronal Medium is compatible with neural progenitor cells (NPCs) generated using several methods, including with STEMdiff™ Neural Induction Medium (Catalog #05835).

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 1.5×10^4 - 6×10^4 cells/cm² in 0.5 mL of the medium in which the cells were maintained. Distribute cells evenly. Incubate at 37°C and 5% CO₂.

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 1.5×10^4 - 3×10^4 cells/cm². For short-term cultures (< 30 days of maturation), seed cells at 4×10^4 - 6×10^4 cells/cm².

2. The next day, add 0.5 mL of Complete Differentiation Medium to the existing culture medium. Incubate at 37°C and 5% CO₂.
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% CO₂ for 2 - 4 weeks, performing a half-medium change (as described in step 3) every 2 - 3 days, 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.

NOTE: For further maturation of NPCs, continue to culture in Complete Differentiation Medium, performing half-medium changes every 2 - 3 days.

NOTE: BrainPhys™ Neuronal Medium is a versatile basal medium that can be used to support the culture of hPSC-derived neurons across various protocols. BrainPhys™ Neuronal Medium can be used for the differentiation of neural progenitor cells and the maturation of neurons. When transitioning from traditional medium (e.g. DMEM/F-12) to BrainPhys™ Neuronal Medium, remove half of the traditional medium from each well and replace it with an equal volume of BrainPhys™ Neuronal Medium, in combination with supplements and cytokines of choice. Continue the culture by performing half-medium changes every 2 - 3 days throughout the duration of the culture.

C. GLUCOSE SUPPLEMENTATION FOR HIGH-DENSITY NEURONAL CULTURES AND NEURAL ORGANOID CULTURES

BrainPhys™ Neuronal Medium contains glucose at the physiological level of 2.5 mM. However, in applications with high cellular metabolic demands, this glucose level may deplete rapidly. To ensure cell survival and to support neuronal activity, supplementing the cultures with additional glucose is recommended. For high-density cultures of primary tissue-derived neurons or hPSC-derived neurons (e.g. in microelectrode array [MEA] experiments where cells are plated at $\geq 1 \times 10^5$ cells/mL), add 12.5 mM glucose to achieve the final concentration of 15 mM in Complete Maturation Medium or Complete Differentiation Medium.

BrainPhys™ Neuronal Medium is a versatile basal medium suitable for the maturation and maintenance of neural organoids, but it should not be used for organoid formation, expansion, or differentiation. If BrainPhys™ Neuronal Medium is used for neural organoid cultures, adding 7.5 mM glucose to reach the final concentration of 10 mM is advised. To transition from other media (e.g. DMEM/F-12 or the basal medium component in either STEMdiff™ Cerebral Organoid Maturation Kit [Catalog #08571] or STEMdiff™ Neural Organoid Maintenance Kit [Catalog #100-0120]), introduce BrainPhys™ Neuronal Medium with glucose during the organoid maturation phase of the protocol. Remove half of the medium from each well and replace it with an equal volume of BrainPhys™ Neuronal Medium with glucose, along with supplements and cytokines of choice (e.g. NeuroCult™ SM1 Neuronal Supplement [Catalog #05711] and N2 Supplement-A). Continue performing full-medium changes every 2 - 4 days until the end of the culture period.

Preparation of Glucose Stock Solution

The following example is for preparing 10 mL of 1M glucose stock solution. If preparing other volumes, adjust accordingly.

1. Add 7 mL of distilled water to a 50 mL tube.
2. Weigh out 1.8 g of D-(+)-glucose (e.g. Sigma G8270) and add to the tube from step 1.
3. Mix to dissolve.
4. Check the volume of the solution and add distilled water to bring the total volume up to 10 mL.
5. Filter sterilize glucose solution and store at 4°C.

NOTE: Sterile 10% w/v D-(+)-glucose solution (e.g. Sigma G8644) can also be used. Dilute 10% w/v D-(+)-glucose solution to 1M solution with distilled water.

References

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

Related Products

For related products, including specialized cell culture and storage media, matrices, antibodies, cytokines, and small molecules, visit www.stemcell.com/hPSCworkflow or contact us at techsupport@stemcell.com.

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