BrainPhys™ Imaging Optimized Medium

Serum-free basal medium supporting neuronal activity and optimized for imaging applications

Catalog #05796 500 mL

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Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
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Product Description

BrainPhys™ Imaging Optimized Medium is a serum-free neuronal basal medium (based on the BrainPhys™ formulation published by Cedric Bardy and Fred H. Gage[1]) that is optimized for imaging applications.

Applications of BrainPhys™ Imaging Optimized Medium include culture of primary neurons, differentiation and maturation of hPSC-derived neurons, and live fluorescent imaging (including calcium imaging and optogenetics). In addition to the removal of phenol red, the formulation has been improved to reduce background fluorescence and increase stability upon repeated exposure to light.

To ensure cell survival in long-term serum-free culture, BrainPhys™ Imaging Optimized Medium must be combined with an appropriate supplement. BrainPhys™ Neuronal Medium and SM1 Kit (Catalog #05792) is recommended for culture of primary neurons; BrainPhys™ Primary Neuron Kit (Catalog #05794) is recommended for plating and culture of primary neurons. BrainPhys™ Neuronal Medium N2-A & SM1 Kit (Catalog #05793) and BrainPhys™ hPSC Neuron Kit (Catalog #05795) are recommended for the differentiation and maturation of hPSC-derived neurons, in combination with lineage-specific growth factors and/or small molecules (if necessary).

Properties

Storage: Store at 2 - 8°C. Protect from light.

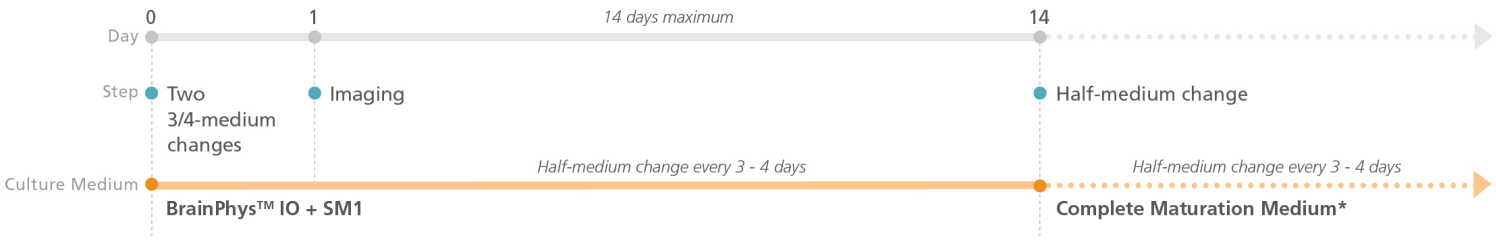
Shelf Life: Stable for 15 months from date of manufacture (MFG) on label.

Directions for Use

Protocols are provided below for A: Primary Tissue-Derived Neurons and B: hPSC-Derived Neurons. Select the appropriate protocol for your cell type.

A. PRIMARY TISSUE-DERIVED NEURONS

Protocol Diagram



*BrainPhys™ Neuronal Medium + NeuroCult™ SM1; refer to the PIS for BrainPhys™

Please read the entire protocol before proceeding.

Preparation of BrainPhys™ Imaging Optimized (IO) + SM1

Use sterile technique to prepare BrainPhys™ IO + SM1 (BrainPhys™ IO 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™ IO Medium (1 in 50 dilution). Mix thoroughly.
   NOTE: If not used immediately, store BrainPhys™ IO + SM1 at 2 - 8°C for up to 1 month. Protect from light.

Culture of Primary Tissue-Derived Neurons

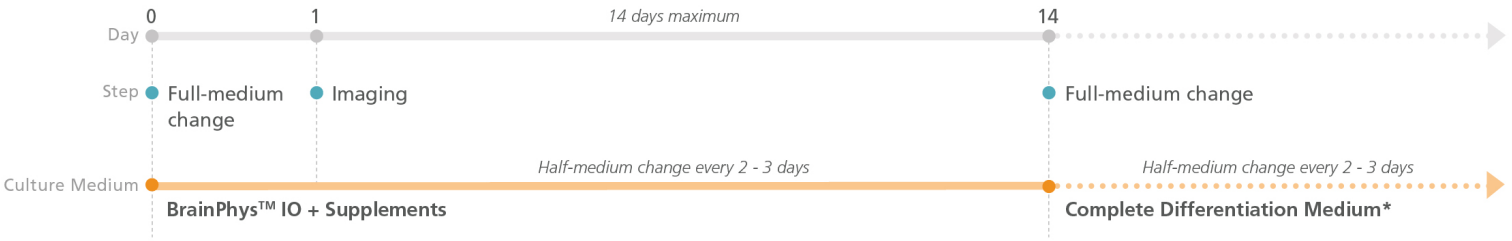
Indicated volumes are for a single well of a 24-well plate. If using other cultureware, adjust volumes accordingly.

1. For culturing primary tissue-derived neurons, refer to the Product Information Sheet (PIS) for BrainPhys™ Neuronal Medium. At Day 5 of the protocol for Culture of Primary Tissue-Derived Neurons, perform one of the following:
   - For further maturation of neurons, perform a half-medium change with Complete Maturation Medium (as described at Day 5). Incubate at 37°C and 5% CO_2. Perform a half-medium change with Complete Maturation Medium every 3 - 4 days. On the day prior to imaging experiments, proceed to step 2.

     OR
   - To change to BrainPhys™ IO + SM1 directly (omit Complete Maturation Medium), proceed to step 2.
2. On the day prior to imaging experiments (**Day 0**), perform two ¾-medium changes with BrainPhys™ IO + SM1, as follows:
   a. Remove ¾ (~0.75 mL) of the medium from each well. Add the same volume of fresh BrainPhys™ IO + SM1 (see Preparation of Media).
   b. Incubate at 37°C for 30 minutes.
   c. Remove ¾ (~0.75 mL) of the medium from each well. Add the same volume of fresh BrainPhys™ IO + SM1 (see Preparation of Media).
   d. Incubate at 37°C for 24 hours.
3. **Day 1**: Perform imaging experiment(s).
4. For extended culture periods, perform a half-medium change with BrainPhys™ IO + SM1 every 3 - 4 days for a **maximum of 14 days**, as follows:
   a. Remove half (~0.5 mL) of the medium from each well.
   b. Add the same volume of fresh BrainPhys™ IO + SM1.
5. **Optional**: To continue culturing neurons after imaging experiments, perform a half-medium change with Complete Maturation Medium (refer to the PIS for BrainPhys™ Neuronal Medium). Incubate at 37°C and perform a half-medium change with Complete Maturation Medium every 3 - 4 days.

B. hPSC-DERIVED NEURONS

Protocol Diagram



*BrainPhys™ Neuronal Medium + Supplements; refer to the PIS for BrainPhys™

Please read the entire protocol before proceeding.

Preparation of BrainPhys™ Imaging Optimized (IO) + Supplements

Use sterile technique to prepare BrainPhys™ IO + Supplements. The following example is for preparing 10 mL of medium. If preparing other volumes, adjust accordingly.

1. Add the following to 10 mL of BrainPhys™ IO Medium:
   - 200 µL of NeuroCult™ SM1 Neuronal Supplement (Catalog #05711)
   - 100 µL of N2 Supplement-A (Catalog #07152)
   - 2 µL of 100 µg/mL Human Recombinant BDNF (Catalog #78005)
   - 2 µL of 100 µg/mL Human Recombinant GDNF (Catalog #78058)
   - 50 µL of 100 mg/mL Dibutyryl-cAMP (Catalog #73882)
   - 7 µL of 50 µg/mL ascorbic acid
2. Mix thoroughly.
   NOTE: If not used immediately, store BrainPhys™ IO + Supplements at 2 - 8°C for up to 2 weeks. Protect from light.

Culture of hPSC-Derived Neurons

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

1. For neuronal differentiation of hPSC-derived neural progenitor cells, refer to the Product Information Sheet (PIS) for BrainPhys™ Neuronal Medium. In the Neuronal Differentiation protocol, perform steps 1 - 3. After a **minimum of 7 days** of culture in Complete Differentiation Medium, proceed to step 2.
2. On the day prior to imaging experiments (**Day 0**), perform a full-medium change with BrainPhys™ IO + Supplements. Incubate at 37°C for 24 hours.
3. **Day 1**: Perform imaging experiment(s).
4. For extended culture periods, incubate at 37°C and perform a half-medium change with BrainPhys™ IO + Supplements every 2 - 3 days for **a maximum of 14 days**, as follows:
   a. Remove half (~0.5 mL) of the medium from each well.
   b. Add the same volume of fresh BrainPhys™ IO + Supplements.
5. **Optional**: To continue culturing neurons after imaging experiments, perform a full-medium change with Complete Differentiation Medium (refer to the PIS for BrainPhys™ Neuronal Medium). Incubate at 37°C and perform a half-medium change with Complete Differentiation Medium every 2 - 3 days.

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 USA 112(20):E2725–34.