# NeuroCult™ Neuronal Basal Medium and NeuroCult™ SM1 Neuronal Supplement

**For the culture of primary neurons**

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|---|---|
| Catalog #05710 | 100 mL |
| Catalog #05711 | 10 mL |
| Catalog #05712 | 1 Kit |

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## Product Description

NeuroCult™ Neuronal Basal Medium (Catalog #05710) is a specialized basal medium designed for the culture of mouse and rat primary neurons. This basal medium must be used in conjunction with an appropriate serum-replacement supplement, such as NeuroCult™ SM1 Neuronal Supplement (NeuroCult™ SM1; Catalog #05711). NeuroCult™ SM1 is a standardized serum-free supplement for the culture of mouse and rat primary neurons and the differentiation and maturation of human embryonic stem cell-/induced pluripotent stem cell-derived neurons. It may be used in combination with BrainPhys™ Neuronal Medium (Catalog #05790) or NeuroCult™ Neuronal Basal Medium. NeuroCult™ SM1 can also be used as a versatile serum-replacement supplement for various applications. The formulation of NeuroCult™ SM1 was developed based on the published formulation of B27[1] supplement, but has been optimized to reproducibly support increased survival and maturation of functional neurons in both short- and long-term cultures.

NeuroCult™ SM1 Neuronal Culture Kit (Catalog #05712) includes NeuroCult™ Neuronal Basal Medium (5 x 100 mL) and NeuroCult™ SM1 Neuronal Supplement (10 mL).

NOTE: NeuroCult™ SM1 is supplied as a 50X concentrate.

### Using NeuroCult™ SM1 with BrainPhys™ Neuronal Medium

NeuroCult™ Neuronal Basal Medium and NeuroCult™ SM1 may also be used for plating primary neurons prior to maturation in BrainPhys™ Neuronal Medium (Catalog #05790). NeuroCult™ SM1 is also a component of the BrainPhys™ Neuronal Medium and SM1 Kit (Catalog #05792) and the BrainPhys™ Neuronal Medium N2-A & SM1 Kit (Catalog #05793). For further details, refer to the Product Information Sheet (PIS) for BrainPhys™ (Document #DX20519), available at www.stemcell.com or contact us to request a copy.

## Product Information

| PRODUCT NAME | CATALOG # | SIZE | STORAGE | SHELF LIFE |
|---|---|---|---|---|
| NeuroCult™ Neuronal Basal Medium | 05710 | 100 mL | Store at 2 - 8°C. | Stable for 8 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. |
| NeuroCult™ SM1 Neuronal Culture Kit<br>• NeuroCult™ Neuronal Basal Medium (5 x 100 mL)<br>• NeuroCult™ SM1 Neuronal Supplement* (10 mL) | 05712 | 1 Kit | | |

*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.

## Materials Required But Not Included

| PRODUCT NAME | CATALOG # |
|---|---|
| L-Glutamine, 200 mM | 07100 |
| L-Glutamic Acid, 2 mg/mL | Sigma G8415 |
| Poly-D-Lysine (PDL) | Sigma P7280 |
| Dulbecco's Modified Eagle's Medium with 4500 mg/L D-Glucose (DMEM High Glucose) | 36250 |
| Falcon® Conical Tubes, 50 mL | 38010 |
| 40 µm Cell Strainer | 27305 |
| Trypan Blue | 07050 |

# Preparation of Complete Medium With and Without L-Glutamic Acid

The following examples are for preparing 10 mL of Complete Medium **With** L-Glutamic Acid and 10 mL of Complete Medium **Without** L-Glutamic Acid. 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 label.
2. Add 0.4 mL of NeuroCult™ SM1 to 19.6 mL of NeuroCult™ Neuronal Basal Medium (1 in 50 dilution).

   NOTE: If not used immediately, store Complete Medium at 2 - 8°C for up to 1 month.
3. Prepare Complete Medium **With** L-Glutamic Acid and Complete Medium **Without** L-Glutamic Acid, as described below.

   Complete Medium **With** L-Glutamic Acid (0.5 mM L-Glutamine and 25 µM [3.7 µg/mL] L-Glutamic Acid)

   Add the following to 10 mL of Complete Medium (prepared in step 2):

   - 25 µL of 200 mM L-Glutamine
   - 18.5 µL of 2 mg/mL L-glutamic acid

   NOTE: This medium is hereafter referred to as Complete Medium **With** L-Glutamic Acid. It is used in tissue dissociation and in the initial plating of primary neurons (sections B and C).

   Complete Medium **Without** L-Glutamic Acid (0.5 mM L-Glutamine)

   Add 25 µL of 200 mM L-Glutamine to 10 mL of Complete Medium (prepared in step 2).

   NOTE: This medium is hereafter referred to as Complete Medium **Without** L-Glutamic Acid. It is used in half-medium changes required during culture of primary neurons (section D).

# Directions for Use

Please read the entire protocol before proceeding.

For the use of NeuroCult™ Neuronal Basal Medium and NeuroCult™ SM1 Neuronal Supplement for plating of primary neurons prior to maturation in BrainPhys™ Neuronal Medium, refer to the PIS for BrainPhys™ (Document #DX20519), available at www.stemcell.com or contact us to request a copy.

A. PREPARATION OF POLY-D-LYSINE (PDL)-COATED CULTURE SURFACE

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

1. Dissolve 5 mg of PDL in 50 mL of sterile water to obtain a 100 µg/mL stock solution.

   NOTE: It is not recommended to prepare the PDL solution with borate buffer. Increased cell clumping is observed when the PDL solution is prepared in borate buffer and cells are then 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.
2. Coat culture wells or glass coverslips with PDL as described below.

   **Coating Culture Wells**

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

   **Coating Glass Coverslips**

   a. Use sterile forceps to place a sterile round glass coverslip in each well of a 24-well plate.
   b. Dilute the 100 µg/mL PDL stock solution (prepared in step 1) with sterile water to 40 µg/mL.
   c. Dispense 0.5 mL of 40 ug/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.
   d. Proceed to step 3.
3. Incubate at room temperature (15 - 25°C) for 2 hours, or wrap the plate with Parafilm® and incubate overnight at 2 - 8°C.
4. Wash each well with 2 x 1 mL of sterile PBS, leaving PBS in the wells.

   NOTE: DMEM/F-12 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.
5. 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. Continue to section B.

B. PREPARATION OF PRIMARY MOUSE OR RAT CNS CELLS FOR CULTURE

NOTE: The procedures described below are for isolation and culture of primary neurons from E14 mouse cortex or E18 rat cortex. Neurons isolated from other central nervous system (CNS) regions or gestational days can also be used. Refer to O'Connor et al.[3] for a detailed procedure for dissecting the cortex.

Tissue may be mechanically dissociated using trituration or enzymatically dissociated with papain, as described below.

### Mechanical Dissociation Using Trituration

1. Dissect and pool cortical tissue in PBS containing 2% glucose (or other appropriate collection medium) in a culture dish.

   NOTE: The amount of tissue dissected and the number of cells needed per experiment will depend on the experiment size. Cortical tissue isolated from the brain of one mouse or one rat embryo will be sufficient to perform the protocol outlined below.
2. Using a glass Pasteur pipette, transfer all of the tissue into a 14 mL tube. Allow the tissue to settle to the bottom, then remove and discard the supernatant. Centrifugation is not necessary.
3. Using a 1 mL pipettor, add 1 mL of Complete Medium **With** L-Glutamic Acid to the tissue.
4. Using the same pipette tip, gently dissociate the tissue by pipetting up and down no more than 5 times, even if chunks or clumps of tissue remain. Be careful not to introduce air bubbles, as this will decrease cell viability.
5. Add 9 mL of DMEM High Glucose to obtain a final volume of 10 mL.
6. Centrifuge the cell suspension at 123 x *g* for 5 minutes. Carefully remove and discard most of the medium.
7. Place a sterile 40 µm Cell Strainer on top of a 50 mL conical tube.
8. Add 1 mL of Complete Medium **With** L-Glutamic Acid to the cell pellet. Pipette up and down 2 - 3 times to resuspend cells.
9. Using a 1 mL pipettor, transfer the cell suspension onto the strainer and allow the cell suspension to flow through the filter.
10. Rinse the filter with an additional 1 mL of Complete Medium **With** L-Glutamic Acid to collect any single cells attached to the filter.
11. Perform a viable cell count using Trypan Blue.
12. Continue to section C for initial plating of primary neurons.

### Enzymatic Dissociation With Papain

1. Prepare a 20 U/mL papain stock solution as follows:
   a. Add 5 mL of DMEM High Glucose or Complete Medium **With** L-Glutamic Acid to each vial of lyophilized papain containing ≥ 100 units (Worthington Catalog #LK003176).
   b. Recap the vial and gently swirl to mix.
   c. Place the vial in a 37°C incubator for 10 minutes.
2. Dissect and pool cortical tissue in PBS containing 2% glucose (or other appropriate collection medium) in a culture dish.

   NOTE: The amount of tissue dissected and the number of cells needed per experiment will depend on the experiment size. Cortical tissue isolated from one mouse brain or one rat embryo will be sufficient to perform the protocol outlined below.
3. Using a glass Pasteur pipette, transfer all of the tissue into a 14 mL tube. Allow the tissue to settle to the bottom and then remove and discard the supernatant. Centrifugation is not necessary.
4. Add 2 mL of papain stock solution (prepared in step 1) to the tissue.
5. Place tube in a 37°C incubator for 5 minutes. Mix the suspension by swirling the tube, then incubate at 37°C for an additional 5 minutes.
6. Using a 1 mL pipettor, carefully remove as much papain stock solution as possible, without disturbing the tissue at the bottom of the tube.
7. Using a 1 mL pipettor, add 1 mL of Complete Medium **With** L-Glutamic Acid. Using the same pipette tip, pipette the tissue up and down 2 - 3 times to triturate. Be careful not to introduce air bubbles.
8. Centrifuge at 650 x *g* for 3 minutes. Carefully remove and discard most of the medium.
9. Place a sterile 40 µm Cell Strainer on top of a 50 mL conical tube.
10. Add 1 mL of Complete Medium **With** L-Glutamic Acid to the cell pellet. Pipette up and down 2 - 3 times to resuspend cells.
11. Using a 1 mL pipettor, transfer the cell suspension onto the strainer and allow the cell suspension to flow through the filter.
12. Perform a viable cell count using Trypan Blue.
13. Continue to section C for initial plating of primary neurons.

C. INITIAL PLATING OF PRIMARY NEURONS

NOTE: Complete Medium **With** L-Glutamic Acid is used in this section. Indicated volumes are for a single well of a 24-well plate. If using other cultureware, adjust volumes accordingly.

1. Resuspend cells with Complete Medium **With** L-Glutamic Acid to obtain a final concentration of 3.9 x 10^6 cells/mL.
2. 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 Medium **With** L-Glutamic Acid.

   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 Medium **With** L-Glutamic Acid.
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. Continue to section D.

D. CULTURE OF PRIMARY NEURONS

NOTE: Complete Medium **Without** L-Glutamic Acid is used in the remainder of the protocol.

1. **Day 0**: Incubate the cultures at 37°C and 5% $CO_2$.
2. **Day 1**: Observe the cells to determine if the cultures are viable (cells are attached with minimal cell debris).
3. **Day 3**: Using a 1 mL pipettor, remove half (approximately 500 µL) of the initial plating medium from wells.
4. Replenish with the same volume of fresh Complete Medium **Without** L-Glutamic Acid. Incubate the cells for an additional 3 days at 37°C and 5% $CO_2$.
5. **Day 6**: The culture should contain a large number of neuroblasts and mature neurons. Mature neurons have extended projections. At this time, most neurons should have projections that are at least twice the length of their cell body.

   NOTE: Cells can now be processed for immunocytochemistry or other applications.
6. For extended culture periods, perform a half-medium change as described in steps 3 - 4 every 3 - 4 days for the remainder of the culture period. Neurons have been cultured for up to 20 days in Complete Medium **Without** L-Glutamic Acid.
7. Observe the cells on a regular basis (e.g. 2 - 3 times per week) to monitor survival of neurons. Neurons should have bright cell bodies and intact axonal projections when viewed using a phase contrast microscope.
8. Upon reaching the end of the desired culture period, cells can be processed for immunocytochemistry or other applications.

For detailed procedures on immunolabeling, refer to the Technical Manual: In Vitro Proliferation and Differentiation of Mouse and Rat Neural Stem and Progenitor Cells Using NeuroCult™ (Document #28704), available at www.stemcell.com or contact us to request a copy.

## References

1. Brewer GJ et al. (1993) Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination. J Neurosci Res 35(5): 567–76.
2. Brewer GJ & Cotman CW. (1989) Survival and growth of hippocampal neurons in defined medium at low density: advantages of a sandwich culture technique or low oxygen. Brain Res 494(1): 65–74.
3. O'Connor TJ et al. (1998) Isolation and propagation of stem cells from various regions of the embryonic mammalian central nervous system. In: Celis JE (Ed.) Cell Biology. A Laboratory Handbook (p. 149). London, UK: Academic Press.