NeuroCult™ Neuronal Basal Medium and NeuroCult™ SM1 Neuronal Supplement

For the Culture of Primary Neurons

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. 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.

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

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

Usage of 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 and N2-A/SM1 Kit (Catalog #05793). For further details, refer to the Product Information Sheet (PIS) for BrainPhys™ (Document #DX20519), available on our website 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.

*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
Trypsin-EDTA	07901
40 µm Cell Strainer	27305
Trypan Blue	07050

Preparation of Complete Neuronal Medium (With and Without L-Glutamic Acid)

The following example is 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.

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

3. a) Add the following to 10 mL of Complete Medium (from 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)

3. b) Add 25 µL of 200 mM L-Glutamine to 10 mL of Complete Medium (from 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 on our website at www.stemcell.com or contact us to request a copy.

A) Preparation of Poly-D-Lysine-Coated Culture Surface

Preparation of 100 µg/mL Poly-D-Lysine (PDL) Stock Solution

1. Dissolve 5 mg of PDL in 50 mL 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 cultured in medium supplemented with NeuroCult™ SM1.

2. Aliquot PDL stock solution into polypropylene vials and store at 2 - 8°C for up to 1 month.

PDL-Coating of Glass Coverslips or Culture Wells

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 the 100 µg/mL PDL stock solution with sterile water to a final concentration of 10 µg/mL PDL.
3. Dispense 0.5 - 1 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 plastic disposable tip to push the coverslip to the bottom of the well.

4. Incubate for 2 hours at 37°C or overnight at 2 - 8°C.
5. At the end of the incubation, wash each well 3 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.
6. The PDL-coated coverslips or wells are ready for use. If not used the same day, store at 2 - 8°C for up to 2 weeks.
7. 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 Prins[3] for a detailed procedure for the dissection of the cortex.

Tissue may be mechanically dissociated using trituration or enzymatically dissociated with Trypsin-EDTA, as described below.

Tissue Dissociation: 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 and then remove and discard the supernatant. Centrifugation is not necessary.
3. Add 1 mL of Complete Medium (with L-Glutamic acid) to the tissue using a 1 mL micropipette.
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 any 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 and resuspend with 1 mL of Complete Medium (with L-Glutamic acid).
7. Place a sterile 40 µm Cell Strainer on top of a 50 mL conical tube.
8. Using a 1 mL micropipette, transfer the cell suspension onto the strainer and allow the cell suspension to flow through the filter.
9. Rinse the filter with an additional 1 mL of Complete Medium (with L-Glutamic acid) to collect any single cells attached to the filter.
10. Perform a viable cell count using Trypan Blue.
11. Continue to section C for initial plating of primary neurons.

Alternate Protocol for Tissue Dissociation: Enzymatic Dissociation with Trypsin-EDTA

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 and then remove and discard the supernatant. Centrifugation is not necessary.
3. Add 3 mL of 0.25% Trypsin-EDTA and mix by gently pipetting up and down.
4. Place tube in 37°C incubator for 15 - 30 minutes.
5. Add 7 mL of DMEM High Glucose with 10% FBS.
6. Centrifuge at 192 x *g* for 1 minute.
7. Remove supernatant, leaving < 1 mL behind.
8. Repeat steps 5 - 7 one more time, for a total of 2 washes.
9. Add 1 mL of DMEM High Glucose and gently triturate the cell suspension by pipetting up and down 3 - 4 times with a 1 mL micropipette. Be careful not to introduce air bubbles.
10. Add 9 mL of DMEM High Glucose to obtain a final volume of 10 mL.
11. Centrifuge at 650 x *g* for 1 minute.
12. Carefully remove most of the medium and resuspend with 1 mL of Complete Medium (**with** L-Glutamic acid).
13. Place a sterile 40 µm Cell Strainer on top of a 50 mL conical tube.
14. Using a 1 mL micropipette, transfer the cell suspension onto the strainer and allow the cell suspension to flow through the filter.
15. Rinse the filter with an additional 1 mL of Complete Medium (**with** L-Glutamic acid) to collect any single cells attached to the filter.
16. Perform a viable cell count using Trypan Blue.
17. Continue to section C.

C) Initial Plating of Primary Neurons

NOTE: Complete Medium (**with** L-Glutamic acid) is used in this section.

The cell density can be adjusted for different applications. It is recommended to plate cells at 1.6 x 10^4 cells/cm^2 (low density) or 4.8 x 10^4 cells/cm^2 (high density).

1. Resuspend cells with Complete Medium (**with** L-Glutamic acid) to obtain a final concentration of 3.9 x 10^6 cells/mL.
2. **To plate cells at 1.6 x 10^4 cells/cm^2**, add 10 µL cell suspension to each 1.3 mL Complete Medium (**with** L-Glutamic acid).
   OR
   To 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. Mix the cells gently using a micropipette set at 1 mL.
4. Plate 1 mL of the mixed cell suspension into a PDL-coated well (or a well containing a PDL-coated coverslip) of a 24-well plate.
5. 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.
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 micropipette 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). Culture the cells for a further 3 days at 37°C.
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 (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 Neural Stem and Progenitor Cells Using NeuroCult™ (Document #28704), available on our website 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. Prins J. (1998) Cell biology. A laboratory handbook. Edited by J.E. Celis. Academic Press.