NeuroCult™ Neuronal Plating Medium

Culture medium for plating dissociated primary tissue-derived neurons for improved survival

Catalog # 05713 100 mL

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

NeuroCult™ Neuronal Plating Medium is a serum-free neuronal basal medium. It is designed for use with BrainPhys™ Neuronal Medium (Catalog #05790) for the plating and culture of primary tissue-derived neurons.

- Optimized for the survival of primary tissue-derived neurons
- Tested for compatibility with BrainPhys™ Neuronal Medium

Properties

Storage: Store at 2 - 8°C.
Shelf Life: Stable for 15 months from date of manufacture (MFG) on label.

Handling / Directions For Use

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: 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.
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 µg/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 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.
   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 phosphate-buffed 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.
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.

PREPARATION OF COMPLETE PLATING MEDIUM
Use sterile techniques to prepare Complete Plating Medium (NeuroCult™ Neuronal Plating 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 NeuroCult™ SM1 to 9.8 mL NeuroCult™ Neuronal Plating Medium (1 in 50 dilution).
3. Add the following supplements and mix thoroughly:
   • 25 µL 200 mM L-Glutamine (Catalog #07100)
   • 18.5 µL 2 mg/mL L-Glutamic acid
   NOTE: If not used immediately, store Complete Plating Medium at 2 - 8°C for up to 1 month.

PREPARATION OF COMPLETE MATURATION MEDIUM

Use sterile techniques to prepare Complete Maturation Medium (BrainPhys™ Neuronal Medium [Catalog #05790] + 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 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. 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% 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 medium from each well. Replenish with the same volume of fresh Complete Maturation Medium.
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.