NeuroCult™ SM1 Without Vitamin A

Supplement (50X) without vitamin A for the culture of neural progenitor cells

Catalog # 05731 10 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™ SM1 Without Vitamin A is a serum-free culture supplement, based on the published B27 formulation, with vitamin A removed. Vitamin A, also known as retinol, induces the differentiation of neural stem cells. In combination with a basal medium of choice, NeuroCult™ SM1 Without Vitamin A can be used for primary and pluripotent stem cell (PSC)-derived neural progenitor cell expansion and in customized differentiation protocols where vitamin A is not desired.

- Vitamin A removed to support neural progenitor and customizable differentiation workflows
- Versatile cell culture supplement
- Optimized, serum-free formulation
- Raw materials rigorously screened to minimize lot-to-lot consistency

Properties

Storage: Store at -20°C.

Shelf Life: Stable until expiry date (EXP) on label.

Contains:
- Antioxidants
- Insulin
- Other ingredients

This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Lot-to-lot variability in color of this product may be expected. This will not affect performance.

Directions For Use

Neural stem and progenitor cells derived from the mouse central nervous system (CNS) can be expanded using neurosphere culture (section B) or adherent culture (section C) methods.

A. PREPARATION OF COMPLETE NEURAL STEM CELL (NSC) MEDIUM

Use sterile technique to prepare complete NSC medium (DMEM/F-12 with 15 mM HEPES + NeuroCult™ SM1 Without Vitamin A + L-glutamine + cytokines).
NOTE: This product does not contain antibiotics. If desired, add penicillin and streptomycin and use medium within 1 week.
The following example is for preparing 500 mL of complete NSC medium. Adjust volumes accordingly if preparing other quantities.

1. Thaw NeuroCult™ SM1 Without Vitamin A at room temperature (15 - 25°C) or overnight at 2 - 8°C.
   NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed expiry date (EXP) on label. Once aliquots are thawed, do not re-freeze.
2. Add 10 mL of NeuroCult™ SM1 Without Vitamin A to 485 mL of DMEM/F-12 with 15 mM HEPES (Catalog #36254) (1 in 50 dilution).
   NOTE: If not used immediately, store at 2 - 8°C for up to 1 month.
3. Immediately before use, supplement medium with the following:
   - 5 mL of 200 mM L-Glutamine (Catalog #07100) to reach a final concentration of 2 mM
   - Human Recombinant EGF (Catalog #78006) to reach a final concentration of 20 ng/mL
   - Human Recombinant bFGF (Catalog #78003) to reach a final concentration of 10 ng/mL
   - 500 µL of Heparin Solution (Catalog #07980) to reach a final concentration of 2 µg/mL (1 in 1000 dilution)

   NOTE: Addition of bFGF and heparin is required only for the culture of neural stem and progenitor cells derived from adult mouse CNS tissues.
   NOTE: Store complete NSC medium at 2 - 8°C for up to 1 week. Do not freeze complete medium.

B. EXPANSION OF NEURAL STEM AND PROGENITOR CELLS IN NEUROSPHERE CULTURES

INITIAL PLATING OF PRIMARY MOUSE CNS CELLS IN NEUROSPHERE CULTURES

1. Plate CNS-derived cells in 10 mL of complete NSC medium (section A) in T-25 cm^2 flasks at the following densities:
   - Embryonic CNS-derived cells: 8 x 10^4 cells/cm^2
   - Adult CNS-derived cells: 2 x 10^4 cells/cm^2
2. Incubate cultures at 37°C in a 5% CO_2 humidified incubator.

HARVESTING CELLS FOR PASSAGING NEUROSPHERE CULTURES

NOTE: Neurospheres should be passaged when they reach 100 - 150 µm in diameter (typically occurs 5 - 8 days after plating). Do not allow neurospheres to grow too large (> 200 µm in diameter); the cells within the core of large neurospheres will lack appropriate gas and nutrient/waste exchange, leading them to necrosis.

1. Harvest and collect the entire cell suspension from the culture into a 15 or 50 mL conical tube (e.g. Catalog #38009 or 38010), depending on the volume harvested.
2. Centrifuge cells at 90 x *g* for 5 minutes. Remove and discard supernatant.
3. Dissociate neurospheres using mechanical dissociation, ACCUTASE™ enzymatic dissociation, or chemical dissociation, as described below.
   a. **Mechanical Dissociation**
      i. Resuspend the cell pellet in 200 µL of complete NSC medium (section A).
      ii. Triturate neurospheres by pipetting up and down with a 200 µL pipettor set to 180 µL, until a single-cell suspension is achieved. Triturate vigorously, but do not introduce air bubbles into the cell suspension.
   b. **ACCUTASE™ Enzymatic Dissociation**
      i. Thaw ACCUTASE™ (Catalog #07920) at 2 - 8°C overnight or at room temperature (15 - 25°C). Do not thaw at 37°C.
      ii. Wet the disposable pipette tip with complete NSC culture medium to prevent cells from sticking to the wall of the pipette tip.
      iii. Add 200 µL of ACCUTASE™ per cell pellet harvested from a T-25 cm^2 flask. Increase the volume of ACCUTASE™ if harvesting from a larger volume. A minimum of 200 µL of ACCUTASE™ is required for dissociation.
      iv. Incubate at room temperature or at 37°C for 5 minutes. At the mid-point of the incubation, mix by gently shaking the tube to ensure the cell suspension is well mixed.
      v. After 5 minutes, determine by eye if there are any undissociated neurospheres or aggregates remaining. If clumps remain, perform a gentle trituration step using a pipettor.
      vi. Wash the cells with 10 mL of complete NSC medium.
      vii. Centrifuge at 150 x *g* for 5 minutes. Discard supernatant.
   c. **Chemical Dissociation**
      Dissociation of neurospheres with NeuroCult™ Chemical Dissociation Kit (Mouse; Catalog #05707) results in high cell viability following dissociation. For more information, refer to the Technical Manual: Chemical Dissociation of Neurospheres Derived from Embryonic and Adult Mouse CNS Using the NeuroCult™ Chemical Dissociation Kit, available at www.stemcell.com or contact us to request a copy.

REPLATING CELLS FOR NEUROSPHERE CULTURES

1. Plate CNS-derived cells in 10 mL of complete NSC medium (section A) in T-25 cm^2 flasks at the following densities:
   - Embryonic CNS-derived cells: 2 x 10^4 cells/cm^2
   - Adult CNS-derived cells: 4 x 10^3 cells/cm^2
2. Incubate cultures at 37°C in a 5% CO_2 humidified incubator.

C. EXPANSION OF NEURAL STEM AND PROGENITOR CELLS IN ADHERENT CULTURES

PREPARATION OF COATED TISSUE CULTURE FLASKS

Tissue culture flasks may be coated with poly-D-lysine (PDL)/laminin or with laminin, as described below.

Preparation of PDL/Laminin-Coated Tissue Culture Flasks

1. Prepare a 100 µg/mL PDL solution by dissolving 5 mg of PDL (Sigma Catalog #P7280) in 50 mL of sterile water.
2. Prepare a 10 µg/mL solution of laminin by diluting the laminin (Sigma Catalog #L2020) in sterile phosphate-buffered saline (PBS) or water.
3. Dispense 3 mL of 100 µg/mL PDL solution into each T-25 cm^2 flask.

4. Incubate at 37°C for 2 hours or 2 - 8°C overnight.
5. Wash each flask with 5 mL of sterile PBS. Remove as much PBS as possible.
6. Dispense 3 mL of 10 µg/mL laminin solution to each T-25 cm^2 flask.
7. Incubate at 37°C for 2 hours or 2 - 8°C overnight.
8. Wash each flask with 5 mL of sterile PBS. Remove the PBS only when ready to plate the cells. Do not let the coated flasks dry completely.
9. The substrate-coated flasks are ready for use and should be used within 2 weeks. Store the substrate-coated flasks at 2 - 8°C until use.

Preparation of Laminin-Coated Tissue Culture Flasks

1. Prepare a 10 µg/mL solution of laminin by diluting the laminin (Sigma Catalog #L2020) in sterile PBS or water.
2. Dispense 3 mL of 10 µg/mL laminin solution into each T-25 cm^2 flask.
3. Incubate at 37°C for 2 hours or 2 - 8°C overnight.
4. Wash each flask with 5 mL of sterile PBS. Only remove the PBS when ready to plate the cells. Do not let the coated flasks dry completely.
5. The substrate-coated flasks are ready for use and should be used within 2 weeks. Store the substrate-coated flasks at 2 - 8°C until use.

INITIAL PLATING OF PRIMARY MOUSE CNS CELLS IN ADHERENT CULTURES

1. Plate CNS-derived cells in 10 mL of complete NSC medium (section A) in PDL/laminin- or laminin-coated T-25 cm^2 flasks at the following densities:
   - Embryonic CNS-derived cells: 8 x 10^4 cells/cm^2
   - Adult CNS-derived cells: 2 x 10^4 cells/cm^2
2. Incubate cultures at 37°C in a 5% CO_2 humidified incubator.
   NOTE: In the presence of a substrate, neural stem and progenitor cells will adhere to the substrate-coated culture vessel within 24 hours. The attached cells have a flattened morphology and are mostly bipolar.

PASSAGING CELLS FOR ADHERENT CULTURES

NOTE: Cultures should be passaged when they reach 60 - 80% confluence.

1. Use a disposable pipette to remove the medium from the culture vessel.
2. Wash cells by adding 5 mL of PBS to each T-25 cm^2 flask. Adjust volume if using other cultureware.
3. Swirl the flask gently. Remove and discard the PBS.
4. Dissociate cells by adding 1 mL of ACCUTASE™ to each T-25 cm^2 flask.
5. Incubate at 37°C for 5 minutes.
6. Observe the culture to determine if the cells are starting to detach or if detachment is complete.
7. Add 5 mL of complete NSC medium using a disposable pipette. Using the same pipette, resuspend and collect the detached cells into a new sterile 15 mL conical tube. If cells remain, add an additional 1 mL of complete NSC medium and repeat the procedure to collect the remaining cells.
8. Centrifuge at 110 x *g* for 5 minutes.
9. Remove all the supernatant and resuspend cells in a maximum of 200 µL complete NSC medium using a 200 µL pipettor set at 180 µL; triturate until a single-cell suspension is achieved.
10. Resuspend cells in an appropriate volume of complete NSC medium and perform a viable cell count using the Trypan Blue (Catalog #07050) dye exclusion method.

REPLATING CELLS FOR ADHERENT CULTURES

1. Plate CNS-derived cells in 10 mL of complete NSC medium in PDL/laminin- or laminin-coated T-25 cm^2 flasks at the following densities:
   - Embryonic CNS-derived cells: 2 x 10^4 cells/cm^2
   - Adult CNS-derived cells: 8 x 10^3 cells/cm^2
2. Incubate cultures at 37°C in a 5% CO_2 humidified incubator.

Related Products

For related products, including specialized culture media, cytokines, dissociation reagents, and cultureware, visit www.stemcell.com/NSPCworkflow or contact us at techsupport@stemcell.com.

References

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.