Applied StemCell, Inc.
(866) 497-4180
www.appliedstemcell.com

Datasheet

Neural Progenitor Cells Culture Protocols

Product Information

Catalog Number

ASM-6005
ASM-6006 (Neural Cellutions Medium)
ASE-5030 (Neural Progenitor Cells)

Handling Instructions

ASM-6016 basal medium is shipped at 4°C and needs to be stored at 4°C upon receipt; at this temperature ASM-6016 basal medium is stable for 1 year post manufacture date. ASM-6016 basal medium is light sensitive; therefore, exposure to light needs to be minimized. ASM-6016-S (growth supplements) is shipped in dry ice and needs to be stored at -20°C upon receipt; at this temperature ASM-6016-S is stable for 1 year post manufacture date.

Quality Control

Medium and supplements undergo sterility testing to assure they are free from bacterial and fungal contamination. The complete medium has been tested and shown to support optimal growth of human cardiomyocytes. In addition, pH, osmolality, endotoxin absence, and other parameters are determined.

Storage Conditions

Store basal medium, reconstituted and complete media in the dark, at 4ºC.

Shelf Life

Supplements are stable at -20ºC for 1 year
Basal medium is stable at 4ºC for 1 year
Reconstituted medium is stable at 4°C for 30 days
Complete medium is stable at 4ºC for 1 week

Materials Required

Materials	Equipment
• Neural Progenitor Cells (ASE-5030) • Neural Cellutions Medium (ASM-6005, ASM-6006) • 1X HBSS (Ca2+/Mg2+free) • Coating Matrix (Recommend Matrigel diluted 1:30 w/DMEM) • Accutase • DNase I • HSA or FBS • FGF-2 (20ng/mL) • EGF (20ng/mL) • PDGF-AA (20ng/mL) • SHH (100ng/mL) • FGF8 (100ng/mL) • GNDF (20ng/mL) • BNDF (20ng/mL) • T3 (40ng/mL) • Ascorbic Acid (200μM) • Retinoic Acid (μM all-trans)	• Incubator • Water Bath • Centrifuge • Biosafety Cabinet • 6-well low binding plate • Culture Vessels • Pipet Tips • Pipets • Conical Tubes • Ice

Applied StemCell, Inc.
521 Cottonwood Dr. #111, Milpitas, CA 95035
Phone: 866-497-4180 (US Toll Free); 408-773-8007 Fax: 408-773-8238
info@appliedstemcell.com www.appliedstemcell.com

Protocol

1. Thawing Cells

1.1 Perform and maintain all cell culture using aseptic techniques.
1.2 Coat culture vessels with matrix of choice (recommended Matrigel) if plating for adherent cells.
1.3 Aliquot and warm only media required. Add fresh EGF and FGF-2
1.4 Thaw cells rapidly and with agitation. It is recommended to thaw neural cells in FBS or HAS rather than media.

Note: Neural Progenitor Cells aggregate as spheres and cannot be accurately counted. Completely dissociating the spheres does not lead to successful propagation.

2. Plating and Maintaining Adherent Cells

2.1 Plate 1 vial of ASE-5030 in neural cellutions medium with fresh growth factors in 1xT75 with coating matrix.
2.2 Feed cells every 2-3 days by performing a complete media change as follows:
- Aspirate and discard medium.
- Wash with warm (37°C) HBSS. Aspirate and discard HBSS.
- Replace with warm neural cellutions medium including fresh growth factors (EGF-FGF-2).

2.3 Visualize cells every day.
2.4 Once cells are 60-70% confluent, change media more regularly (every other day).
2.5 Once cells are nearing confluency (~80%), prepare vessels for passage/expansion.

Note: Do not let cells become overconfluent.

3. Plating and Maintaining Suspension Cells

3.1 Plate 1 vial of ASE-5030 in neural cellutions medium with fresh growth factors in 1-2 wells of a 6-well low binding plate.
3.2 Feed cells every 2-3 days by performing a complete media change as follows:
- Carefully remove medium with spheres to a conical tube.
- Pipet spheres with a P200 to break up, but do not completely dissociate.
- Spin conical tube at 400g for 5 minutes at 4°C.
- Carefully aspirate supernatant and resuspend pellet in warmed neural cellutions medium with fresh growth factors(EGF/FGF-2).

  Note: See Step 6 for additional growth factor additives for differentiation.

- Return newly suspended cells to original low binding wells.

4. Passing of Adherent Cells

4.1 Aspirate and discard medium.
4.2 Wash with warm (37°C) HBSS. Aspirate and discard HBSS.
4.3 Add enzyme solution (recommended Accutase with DNase I).

Note: Observe the detachment of cells. Trypsin is not recommended. If using Trypsin, observe detachment every 1-2 minutes as Trypsin has toxic effects on neural cells.

4.4 Add 10% FBS or HSA to enzyme solution and transfer from flask to conical tube.
4.5 Wash flask with warm HBSS and add to conical tube.
4.6 Centrifuge cell suspension at 400g for 5 minutes at 4°C.
4.7 Carefully aspirate supernatant and resuspend cells in warm neural cellutions medium for counting.

5. Passing of Suspension Cells

5.1 Carefully remove medium with spheres to a conical tube.
5.2 Wash wells with HBSS to insure that all spheres were collected.
5.3 Spin conical tube at 150xg (1200rpm) for 5 minutes at 4°C.
5.4 Remove supernatant and add 2-3 mL of Accutase with DNase I.
5.5 Triturate gently using a pipetman with a P1000 tip followed by a P200 tip – Avoid producing bubbles.

5.6 Incubate for 5-10 minutes at 37°C.
5.7 Triturate again.
5.8 Add 10% FBS and spin at 150xg (1200rpm) for 5 minutes at 4°C.
5.9 Passage cells 1:2 to 1:3 in warmed Neural Cellutions Medium with fresh growth factors (EGF/FGF-2).

Note: Do not let spheres get too large as this causes internal cell death.

6. Differentiation of Neural Progenitor Cells

6.1 For Glial Cells:
- Maintain cells in PDGF-AA, FGF-2, T3 and EGF for no less than 2 weeks.
- Change media and add fresh growth factor every 2-3 days.

6.2 For Neuronal Cells:
- Maintain cells in Shh, FGF-2 and FGF8 for no less than 2 weeks.
- Change media on cells with fresh growth factor every 2-3 days.

6.3 For Both Cell Types:
- After a minimum of 2 weeks, add fresh all trans retinoic acid (RA) for 3 consecutive days with fresh media changes each day – RA is light sensitive.
- Continue adding growth factor from previous step with RA.

6.4 On Day 4, remove RA from media and plate spheres in matrix coated flask/plate. If cells are already adherent, proceed to next step.

6.5 For Glial Cells, continue only with EGF and remove PDGF-AA, FGF-2 and T3.

6.6 For Neuronal Cells, discontinue SHH, FGF-2, FGF8 and add GDNF, BDNF and ascorbic acid. Leave cells on terminal differentiation for a minimum of 21 days.

Note: If cells become overly confluent, passage to freshly coated culture vessel.