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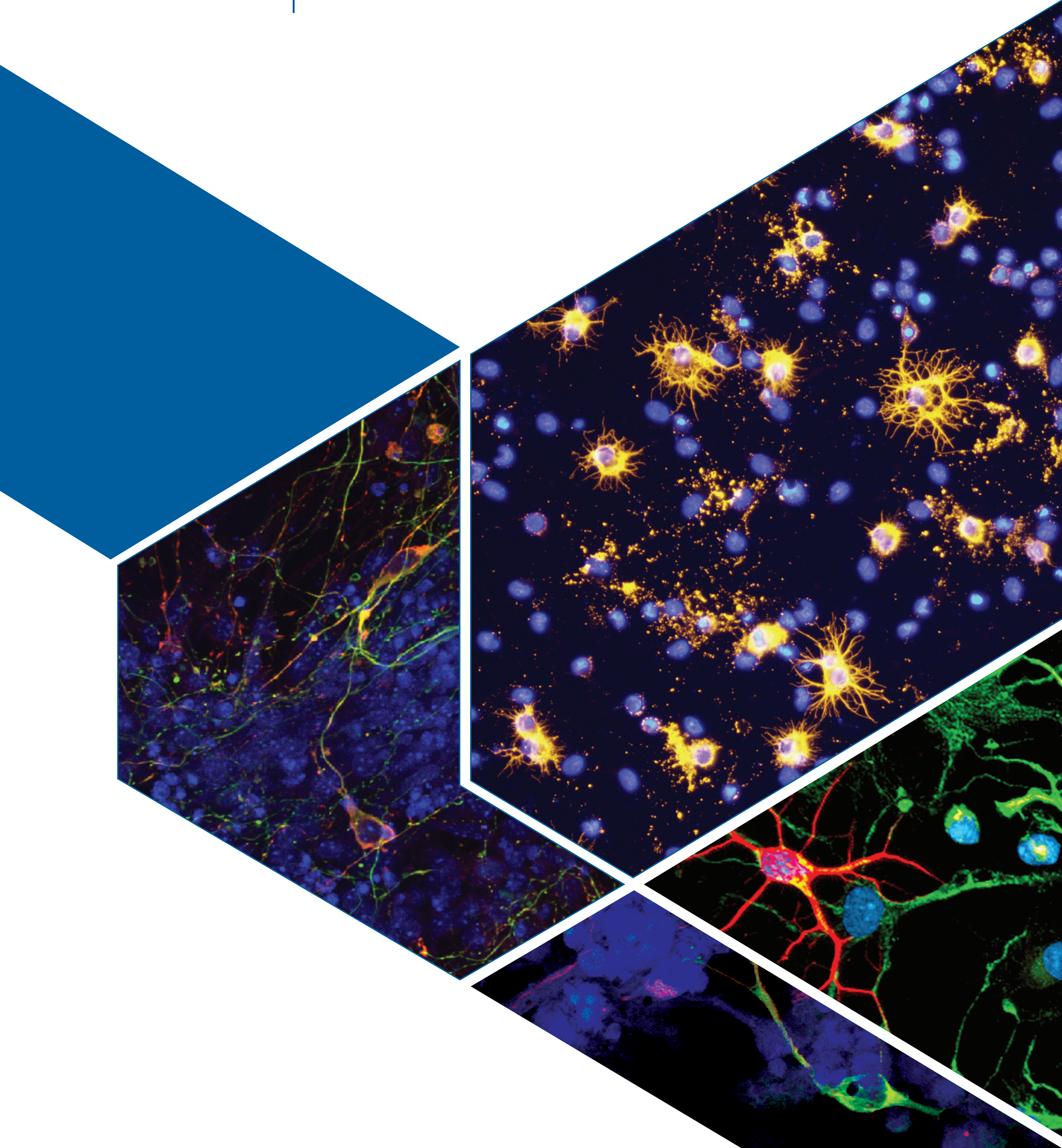
Neural Cell Culturing Guide



The advent of *in vitro* culturing of neural cells has been central to driving our understanding of the nervous system. The complexity of neural tissue makes it difficult to study the cellular and molecular mechanisms underlying neural functioning. Cell culture systems have given researchers the means to study the nervous system at this level. This guide provides an introduction to the techniques and protocols for successfully isolating and culturing specific cell types from the rodent central and peripheral nervous systems, as well as the expansion of mouse and rat cortical stem cells. Contributors include Bio-Techne scientists from our bioassay and stem cell divisions, who have experience in culturing and maintaining multiple types of neural cells. This guide also presents the industry's premiere media supplements, adhesion substrates, bioactive proteins, verification antibodies, and neuromodulatory compounds from the Bio-Techne brands R&D Systems, Tocris, and Novus Biologicals for neural cell culture research.

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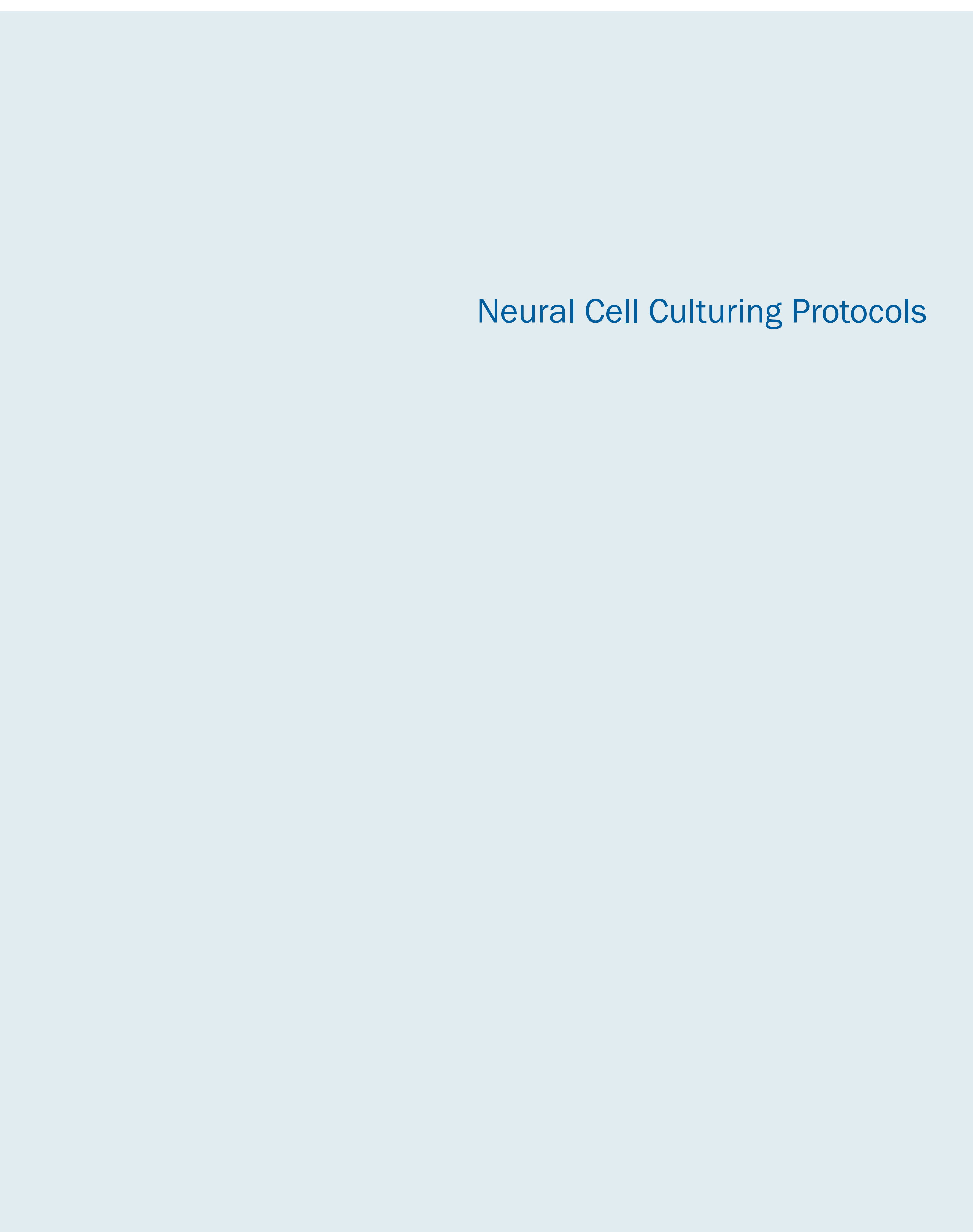


Neural Cell Culturing Protocols

Protocol for Culturing Embryonic Rat Spinal Motor Neurons

The spinal motor neuron culture is an indispensable model system for studying neuronal development, regeneration, and the mechanisms underlying motor neuron diseases such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). Motor neuron survival *in vitro* requires a specific combination of multiple growth factors and supplemental reagents. This protocol describes the use of these growth factors and supplemental reagents for successful culture of motor neurons *in vitro* together with a simple step-by-step method for isolating and culturing these neurons.

Note: Aseptic techniques should be used in this protocol to ensure there is no bacterial, fungal, or mycoplasma contamination. The initial dissection and collection of the spinal cords can be completed outside of a laminar flow cell culture hood. However, preparation of cell culture plates and all steps following tissue harvest should be conducted within a hood. Likewise, all reagents and materials used should be sterile.

Supplies Required

Reagents

Note: *Cold Spring Harbor Protocols* provides a recipe for the SATO supplement.[1] The N-2 MAX Media Supplement (R&D Systems, Catalog # AR009) offers a serum-free alternative to the SATO supplement.

Note: Insulin has low solubility at a neutral pH. Dilute HCl acid (e.g. 1 N) can be added to the solution to help solubilize Insulin.

- N-acetyl-L-cysteine solution (5 mg/mL in Neurobasal® medium)
- Bovine Serum Albumin (BSA, 4% in DPBS)
- Cultrex® Mouse Laminin I (R&D Systems, Catalog # 3400-010-01)
- Cultrex® Poly-D-Lysine (R&D Systems, Catalog # 3439-100-01)
- Deionized or distilled H_2O, sterile (dH_2O)
- Dimethyl sulfoxide (DMSO)
- DPBS, no Ca^{2+}, no Mg^{2+} (ThermoFisher Scientific, Catalog # 14190250), or equivalent
- Fetal bovine serum (FBS)
- Forskolin solution (4.2 mg/mL in DMSO)
- L-glutamine-penicillin-streptomycin solution (100x), or equivalent
- Insulin solution (0.5 mg/mL in dH_2O)
- Isobutylmethylxanthine solution (1 mg/mL in DMSO)
- Leibovitz's L-15 Medium (ThermoFisher Scientific, Catalog # 14415064), or equivalent
- N21-MAX Media Supplement (50x, R&D Systems, Catalog # AR008)
- NaOH (0.1 N)
- Neurobasal® medium (ThermoFisher Scientific, Catalog # 21103049), or equivalent
- OptiPrep™ Density Gradient Medium (Sigma-Aldrich, Catalog # D1556), or equivalent
- Recombinant Human/Mouse/Rat/Canine/Equine BDNF (R&D Systems, Catalog # 248-BD)
- Recombinant Human CNTF (R&D Systems, Catalog # 257-NT)
- Recombinant Human GDNF (R&D Systems, Catalog # 212-GD)
- SATO supplement (100x), or equivalent
- Sodium pyruvate (100 mM)
- 3,3,5-triiodo-L-thyronine (T3) sodium salt solution (4 µg/mL)
- Trypan blue (0.4%)
- TrypZean™ solution (1x, Sigma-Aldrich, Catalog # T 3449), or equivalent

Optional Reagent:

- Cytosine arabinoside (Sigma-Aldrich, Catalog # C1768)

Materials

- 0.22 µm sterile filter unit
- 15 mL conical centrifuge tubes, sterile
- 50 mL conical centrifuge tubes, sterile
- Cell culture plates (24-well), sterile
- E14–E15 timed pregnant rat
- Ice
- Parafilm®
- Pasteur pipette, glass, fire-polished, sterile
- Petri dishes, 60 × 15 mm
- Petri dishes, 100 × 20 mm
- Pipette tips
- Sylgard®-lined dissection petri dish, 93 × 22 mm (Living Systems Instrumentation, Catalog # DD-90-S-BLKO), or equivalent
- Syringe filters, 0.22 µm (Cole-Parmer, Catalog # UX-81053-14), or equivalent

Equipment

- 37 °C, 5% (or 10%) CO_2 humidified incubator
- 37 °C water bath
- Autoclave
- Centrifuge
- Dissecting microscope
- Dissection pins, 0.1 mm diameter (Living Systems Instrumentation, Catalog # PIN-0.1MM), or equivalent
- Dissection tools
    - Dumont, #5, mirror finish forceps (quantity 2)
    - Fine forceps, #5, straight
    - Fine scissors, ToughCut®
    - Graefe forceps
    - Surgical scissors, small
    - Vannas-Tübingen spring scissors, 2.5 mm cutting edge
- Hemocytometer
- Inverted microscope
- Laminar flow cell culture hood
- Pipettes

Reagent Preparation

Note: All prepared solutions should be sterile filtered in a laminar flow cell culture hood prior to addition to the medium.

T3 Solution (4 µg/mL)

1. Prepare an 8 mg/mL T3 solution in 0.1 N NaOH.
2. Add 10 µL of the 8 mg/mL T3 solution to 20 mL of DPBS
3. Filter using a 0.22 µm syringe filter to sterilize.

Culture Media

1. Add the following reagents to the Neurobasal® medium at the given final concentration.

Reagent	Final Concentration
N21-MAX Media Supplement (50x)	1x
SATO supplement (100x)	1x
Insulin solution (0.5 mg/mL)	5 µg/mL
Sodium pyruvate (100 mM)	1 mM
L-glutamine-penicillin-streptomycin solution (100x)	1x
T3 solution (4 µg/mL)	40 ng/mL
Cultrex® Mouse Laminin I	1 µg/mL
Isobutylmethylxanthine solution (1 mg/mL)	2.2 µg/mL
Forskolin solution (4.2 mg/mL)	0.42 µg/mL
N-acetyl-L-cysteine solution (5 mg/mL)	5 µg/mL

2. In a laminar flow cell culture hood, sterile filter the solution using a 0.22 µm sterile filter unit.
3. Add the following growth factors to the solution at the given final concentration.

Growth Factor	Final Conc.
Recombinant Human/Mouse/Rat/ Canine/Equine BDNF	10 ng/mL
Recombinant Human CNTF	10 ng/mL
Recombinant Human GDNF	10 ng/mL

Procedure

Coating of Cell Culture Plates

Note: Preparation of the cell culture plates should be done in a laminar flow cell culture hood.

1. Dilute the Cultrex® Poly-D-Lysine solution with sterile dH_2O to a final concentration of 50 µg/mL.
2. Add 200 µL of the 50 µg/mL Cultrex® Poly-D-Lysine solution to each well of the cell culture plates. Tilt the plates gently to ensure even coating of the well surface.
3. Incubate plates for 1 hour in a 37 °C, 5% CO_2 humidified incubator.
4. Aspirate the poly-D-lysine solution. Wash the wells three times with sterile dH_2O. After the third wash, aspirate the wells to completely remove all liquids.
5. Wrap plates with parafilm® to seal. Store plates at 2–8 °C for up to 2 weeks.

Note: Alternatively, spinal motor neurons can be cultured on pre-coated glass coverslips, such as mouse Laminin I and poly-D-lysine coated glass coverslips, 12 mm diameter, # 1.5 thickness (neuVitro, Catalog # GG-12-1.5-laminin), or equivalent. Place one coverslip into each well of a 24-well cell culture plate.

Dissection of Embryonic Rat Spinal Cords

Note: Autoclave dissection tools to sterilize.

1. Warm an appropriate amount of media in a 37 °C water bath.
2. Place sterile DPBS on ice.
3. Asphyxiate pregnant rat with CO_2. Recover embryos via cesarean section using the fine scissors and Graefe forceps. Place the embryos in a 100 × 20 mm petri dish containing cold DPBS. Keep the dish on ice.
4. Remove the embryos from their individual placenta sacs and wash with cold DPBS.
5. Place cleaned embryos in a new 100 × 20 mm petri dish containing cold DPBS. Carefully decapitate each embryo at the head/neck junction using a small surgical scissors. Discard the heads.
6. Place the body of the embryo with the ventral side down in a 93 × 22 mm Sylgard®-lined dissection petri dish. Position the embryo so that the tail is pointed towards you. Place a 0.1 mm dissection pin through each limb to fasten the embryo to the dish.
7. With the ToughCut® fine scissors, cut off the tail. Under a dissecting microscope, carefully remove skin and tissue with the #5 fine forceps, moving in a ventral direction, until the dorsal surface of the spinal cord is visible.
8. Moving caudal to rostral, cut along the dorsal midline of the spinal cord with the Vannas-Tübingen spring scissors to "open" the spinal cord, separating the left and right sides.
9. Use the Dumont, #5, mirror finish forceps to remove the extraneous tissue that surrounds the spinal cord, exposing the dorsal root ganglia (DRG). Rub the Dumont, #5, mirror finish forceps between the spinal cord and DRG and meninges to remove these tissues. Repeat this process on the other side to free the spinal cord.
10. Remove the spinal cord from the body by grapping onto one end of the spinal cord and lifting it up and away from the body. Use the Vannas-Tübingen spring scissors to cut along the dorsal-ventral midline to trim off the dorsal part of the spinal cord.
11. While holding the remaining ventral spinal cord, cut along the ventral midline to bisect it.
12. Transfer the isolated ventral spinal cord to a clean 60 × 15 mm dish containing cold L15 medium. Mince the spinal cord into small pieces with the Vannas-Tübingen spring scissors.

Dissociation and Culture of Embryonic Rat Spinal Motor Neurons

Note: From this point forward, any opening of tubes/plates that contain any tissue, cells, media, or reagents should be done in a laminar flow cell culture hood.

1. Transfer the dissected spinal cord tissue and L15 medium to a 15 mL conical tube. Centrifuge at 193 × g for 3 minutes at room temperature. Decant the medium from the pelleted tissue pieces.
2. Add 3 mL of the TrypZean™ solution, diluted 1:1 in sterile DPBS, to the pelleted tissue. Incubate for 15 minutes in a 37 °C water bath, agitating every 3 minutes.
3. Add 3 mL of FBS to the 15 mL conical tube. Centrifuge at 193 × g for 3 minutes at room temperature. Decant the solution from the pelleted tissue pieces.
4. Coat a fire-polished Pasteur pipette with FBS.
5. Add 6 mL of L15 medium to the 15 mL conical tube. Gently triturate the tissue pieces with the fire-polished Pasteur pipette until the solution is homogenous.

Note: Avoid generating bubbles while triturating.

6. Prepare a 9% OptiPrep™ solution in L15 medium. Transfer 3 mL of the solution to each of six 15 mL conical tubes.
7. Divide the homogenized solution evenly among the six tubes containing the OptiPrep™ solution. Centrifuge the tubes at 430 × g for 15 minutes at room temperature.

Note: Brakes should be off during this centrifugation step.

8. Carefully collect the top 2 mL of solution from each tube and pool into a 50 mL conical tube. Fill the 50 mL conical tube with L15 medium. Centrifuge at 193 × g for 5 minutes at room temperature. Decant the solution from the pelleted cells.
9. Resuspend the cells in 6 mL of L15 medium. Transfer the cell suspension to a new 15 mL conical tube containing 1 mL of 4% BSA. Slowly layer the cell suspension on top of the 4% BSA solution. Centrifuge at 260 × g for 10 minutes at room temperature. Decant the solution from the pelleted cells.

10. Resuspend the cells in 250–500 µL of culture media. Mix 10 µL of the cell suspension with 10 µL of 0.4% Trypan blue. Count the live cells.
11. Cover the previously prepared coverslips with 100 µL of culture media per coverslip. Add 10–20 µL of the cell suspension to each coverslip.

Note: Dissociated cells are plated at a low density (~ 25–50 cells/mm^2).

12. Incubate the cell culture plates in a 37 °C, 5% CO_2 humidified incubator for at least 2 hours to allow the cells to adhere to the coverslips.
13. Carefully add 900 µL of culture media to each well of the cell culture plate.
14. Keep cultured spinal motor neurons in a 37 °C, 5% CO_2 humidified incubator until use.

Note: If interested in immunopanning to increase the purity of the motor neuron cultures, refer to Graber, D.J. and B.T. Harris[1] for more information.

Exchanging Media in Spinal Motor Neuron Cultures

Note: For long-term cultures, add 100 nM of cytosine arabinoside to the spinal motor neuron cultures on days 4 and 8 to reduce the proliferation of residual astrocytes. Healthy cultures can be maintained for up to 4 weeks.

1. Warm an appropriate amount of culture media in a 37 °C, 5% CO_2 humidified incubator.
2. Gently remove half the volume of media (i.e. 500 µL) from each well of the cell culture plates. Gently add 500 µL of new, warmed culture media to each well of the cell culture plates.

Note: Do not remove all the media from the wells of the cell culture plate as this will stress the spinal motor neurons.

3. Exchange the culture media every 3–4 days.

References

1. Graber, D.J. and B.T. Harris (2013) Cold Spring Harb. Protoc. **2013**:319.
2. Leach, M.K. *et al.* (2011) J. Vis. Exp. **48**:2389.
3. Fantetti, K.N. and D.M. Fekete (2011) J. Vis. Exp. **58**:3600.

Protocol for Culturing Embryonic Rat Spinal Motor Neurons

Day 1

Prepare cell culture plates by coating with Poly-D-Lysine.

Day 2

Recover E14–15 rat embryos. **Decapitate.**

small surgical scissors

continued on next line

Place rat embryos dorsal side up in dissection dish. **Expose** dorsal spinal cord by removing skin and tissue. **Open** the spinal cord by cutting along the dorsal midline.

Vannas-Tübingen spring scissors

dorsal midline

Sylgard®-lined dissection dish

Continue to **remove** tissue to expose the dorsal root ganglia (DRGs). **Separate** DRGs from both sides of the spinal cord.

Dumont #5 forceps

DRGs

spinal cord

Remove isolated spinal cords. **Trim** off the dorsal column and **cut** into small pieces.

spinal cord

Transfer dissected spinal cord tissue. **Centrifuge. Decant** supernatant.

Centrifuge @ 193 × g 3 minutes, room temperature

L15 Medium

15 mL conical tube

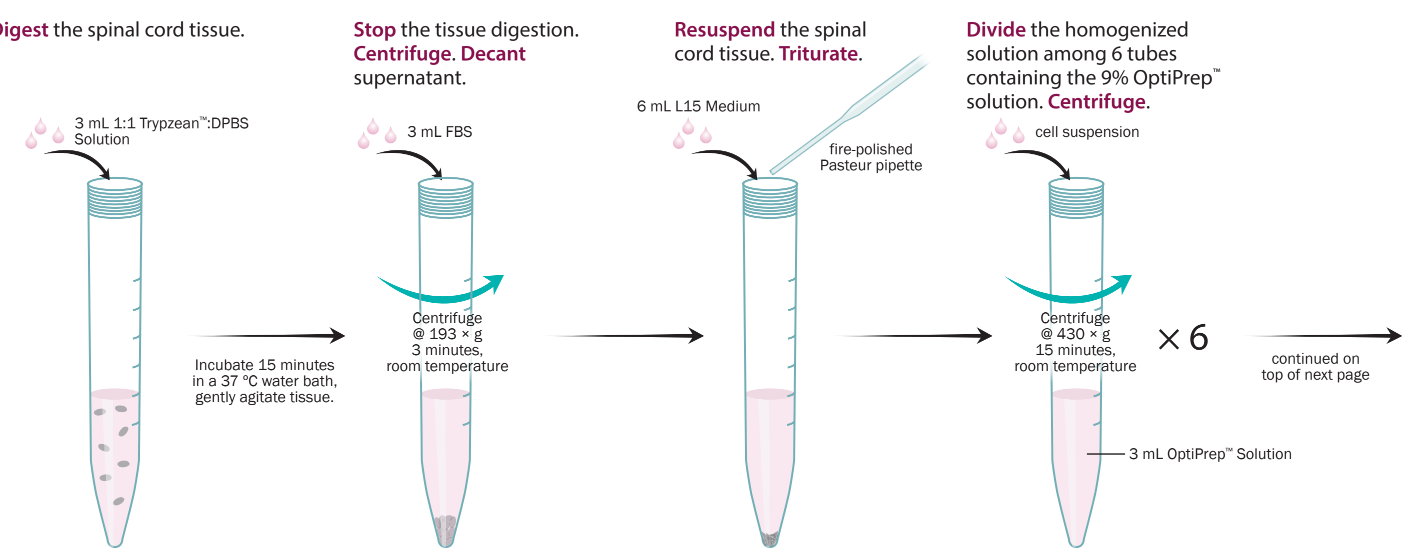
continued on next line

Digest the spinal cord tissue.

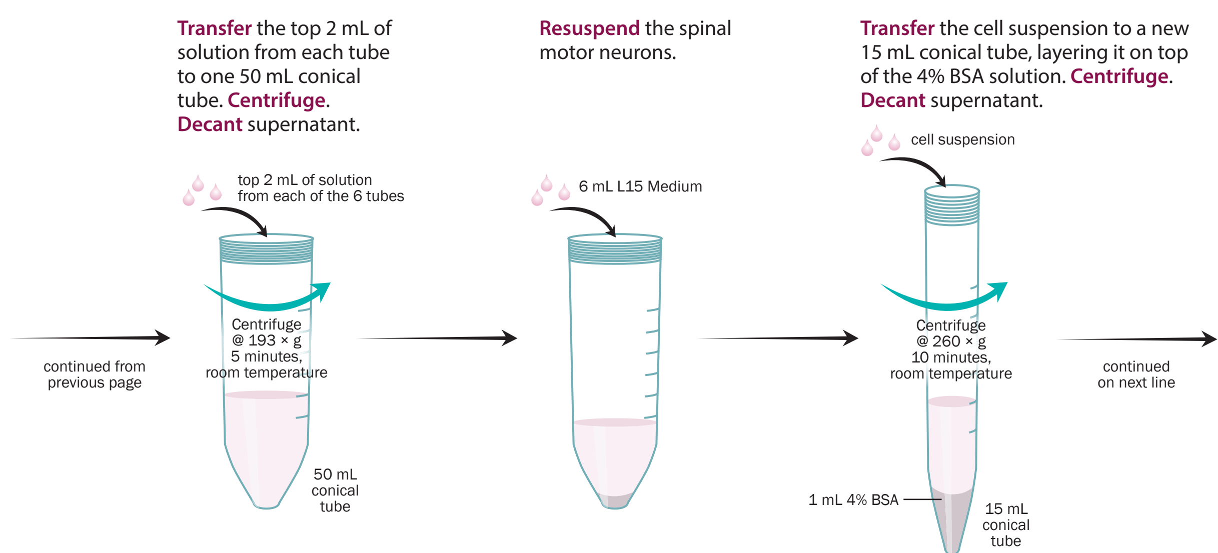
Stop the tissue digestion. **Centrifuge. Decant** supernatant.

Resuspend the spinal cord tissue. **Triturate.**

Divide the homogenized solution among 6 tubes containing the 9% OptiPrep™ solution. **Centrifuge.**



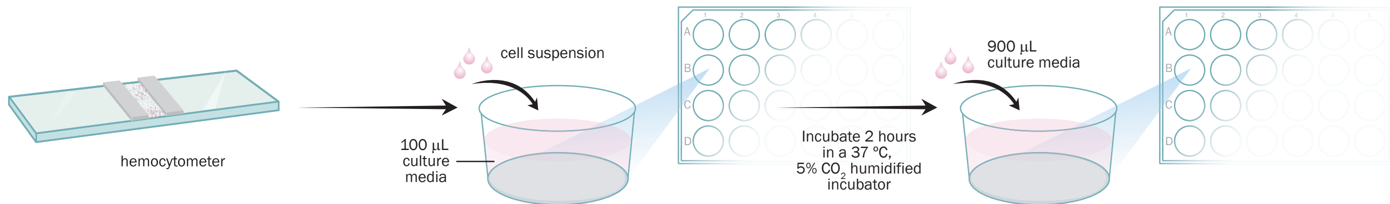
Protocol for Culturing Embryonic Rat Spinal Motor Neurons, continued



Resuspend the spinal motor neurons in 250–500 µL of culture medium. **Count** cells.

Seed neurons onto Poly-D-Lysine-coated cell culture plates.

Add culture media to each well of the plate. **Culture** spinal motor neurons for desired amount of time. **Exchange** media every 3–4 days.



Protocol for Culturing Embryonic Chick Dorsal Root Ganglion Neurons

Dorsal root ganglion (DRGs) neurons are somatosensory neurons that reside in ganglions on the dorsal root of the spinal cord. Chick DRG culture is an indispensable model system for studying neurite outgrowth, regeneration, and degeneration, as well as the molecular mechanisms of nociception and myelination in the central and peripheral nervous systems. This protocol provides step-by-step instructions for dissecting and culturing a semi-pure DRG culture.

Please read the protocol in its entirety before starting.

Note: Aseptic techniques should be used in this cell culture protocol to ensure there is no bacterial, fungal, or mycoplasma contamination. The initial dissection and collection of DRGs can be completed outside of a laminar flow cell culture hood. However, preparation of reagents and cell culture plates, and all steps following tissue harvest should be conducted within a hood. Likewise, all reagents and materials used should be sterile.

Supplies Required

Reagents

- 70% ethanol
- Cultrex® Mouse Laminin I (R&D Systems, Catalog # 3400-010-01) or Bovine Fibronectin Protein (R&D Systems, Catalog # 1030-FN)
- DME medium, high glucose, no L-glutamine (Irvine Scientific, Catalog # 9024), or equivalent
- Fetal bovine serum (FBS)
- L-glutamine-penicillin-streptomycin solution (100x), or equivalent
- Ham's F-12K (Kaighn's) medium
- HEPES buffer solution (1 M)
- N-2 MAX Media Supplement (100x, R&D Systems, Catalog # AR009)
- PBS, sterile (1x): 0.137 M NaCl, 0.05 M NaH_2PO_4, pH 7.4
- Recombinant Human β-NGF (R&D Systems, Catalog # 256-GF)
- Sodium pyruvate (100 mM)
- Trypan blue (0.4%)
- Trypsin solution (10x, Sigma-Aldrich, Catalog # T4549), or equivalent

Materials

- 15 mL conical centrifuge tubes, sterile
- Alcohol pads
- Cell culture plates (96-well), sterile
- E10–E13 embryonated Leghorn chicken eggs
- Ice
- Paper towel
- Pasteur pipette, glass, fire-polished, sterile
- Petri dishes, 60 × 15 mm
- Petri dishes, 100 mm
- Pipette tips

Equipment

- 37 °C, 5% CO_2 humidified incubator
- 37 °C water bath
- Centrifuge
- Dissecting microscope
- Dissection tools
  - Dissecting forceps, straight (e.g. Gillies)
  - Fine forceps, #5, straight
  - Fine forceps, #5/45, angled 45°
  - Forceps, #7, curved
  - Scissors, extra narrow, straight, sharp tip
- Hemocytometer
- Inverted microscope
- Laminar flow cell culture hood
- Pipettes

Reagent Preparation

Note: Prepare all solutions in a laminar flow cell culture hood.

Dissection Media

1. 1x L-glutamine-penicillin-streptomycin solution, 20 mM HEPES buffer solution in Ham's F-12K medium

Plating Media

1. 1x L-glutamine-penicillin-streptomycin solution, 1 mM sodium pyruvate, 10 mM HEPES buffer solution, 10% FBS in a Ham's F-12K medium/DMEM (1:1) solution

Culture Media

1. 1x L-glutamine-penicillin-streptomycin solution, 1 mM sodium pyruvate, 10 mM HEPES buffer solution, 1x N-2 Plus Media Supplement, 1 ng/mL Recombinant Human β-NGF in a Ham's F-12K medium/ DMEM (1:1) solution

Procedure

Coating of Cell Culture Plate

Note: Preparation of the cell culture plates should be done in a laminar flow cell culture hood.

1. Prepare a 15 μg/mL solution of either Mouse Laminin I or Bovine Fibronectin Protein in sterile PBS.
2. Add 50 μL of the solution to each well of a cell culture plate. Incubate the plate overnight at 2–8 °C.
3. Wash the wells twice with 100 μL/well of sterile PBS. Add 50 μL of culture media to each well of the cell culture plate. Incubate the plate in a 37 °C, 5% CO_2 humidified incubator for 30 minutes. Plate is ready for DRG neurons to be seeded.

Dissection of Embryonic Chick DRGs

Note: Soak dissection tools in 70% ethanol for 20–30 minutes to sterilize. Place the tools on a paper towel and let air dry before use.

1. Place sterile PBS and dissection media on ice.
2. Wipe an embryonated egg with an alcohol pad.
3. Holding the egg with the air sack on top, crack the eggshell. Pull the chicken embryo out using the dissecting forceps and place in a 60 × 15 mm petri dish. Decapitate the embryo using the extra narrow scissors and discard the head.
4. With the embryo on its back, clear the spinal cord of all the visceral tissues and organs using the #7 curved forceps. Rinse the embryo cavity with sterile PBS and then dissection media.
5. Under a dissecting microscope, dissect out the DRGs all along the spinal cord using the #5 fine forceps and place in a 60 × 15 mm petri dish containing fresh, cold dissection media. Use the #5 fine forceps and the #5/45 fine forceps to remove the extraneous tissues from the isolated DRGs.

Note: Do not puncture the outside membrane of the DRGs.

6. Transfer the clean DRGs to a separate 15 mL conical tube containing cold dissection media. Keep DRGs on ice until dissection is complete.
7. Repeat steps 3–7 for 4 to 7 chicken embryos, which should provide enough DRG neurons for one 96-well cell culture plate.
8. After all DRGs have been isolated, centrifuge the 15 mL conical tube at 193 × g for 3–5 minutes at room temperature.

Note: From this point forward, the opening of tubes/plates that contain any tissue, cells, media, or reagents should be done in a laminar flow cell culture hood.

9. Discard the supernatant.

Dissociation and Culture of Embryonic Chick DRGs

1. Warm an appropriate amount of plating and culture media in a 37 °C, 5% CO_2 humidified incubator.
2. Resuspend the DRGs in 4.8 mL of sterile PBS. Add 200 µL of 2.5% Trypsin with no EDTA. Mix the contents of the 15 mL conical tube by gentle agitation. Incubate the tube in a 37 °C water bath for 4–10 minutes, gently agitating the tube several times during the incubation.

Note: The length of this trypsinization step will vary depending on the number of DRGs that were isolated. The incubation period is over once the DRGs clump together, at which point, the 15 mL conical tube can be removed from the 37 °C water bath.

3. Add 7 mL of plating media to the 15 mL conical tube. Centrifuge at 193 × g for 3–5 minutes at room temperature. Discard the supernatant.
4. Resuspend DRGs in 4–5 mL of plating media. Dissociate the DRG tissue into a single cell suspension by trituration with the fire-polished Pasteur pipette.
5. Add 8–9 mL of plating media. Transfer the cell suspension to a 100 mm petri dish. Incubate the dish for 3–4 hours in a 37 °C, 5% CO_2 humidified incubator.

Note: During this incubation, non-neuronal cells will attach to the bottom of the petri dish while the DRG neurons will remain in suspension.

6. Collect and transfer the plating media (hence, the non-attached DRG neurons) to a 15 mL conical tube. Centrifuge at 193 × g for 5–7 minutes at room temperature. Discard the supernatant.
7. Resuspend the neurons in 2–5 mL of culture media. Mix 10 µL of the cell suspension with 10 µL of 0.4% Trypan blue. Count the live cells.
8. Reconstitute the cells with culture media to a concentration of 15–20 × 10^4 cells/mL. Add 100 µL of the cell suspension to each well of a prepared cell culture plate so there are 15,000–20,000 cells/well.
9. Keep the cultured DRG neurons in a 37 °C, 5% CO_2 humidified incubator until use.

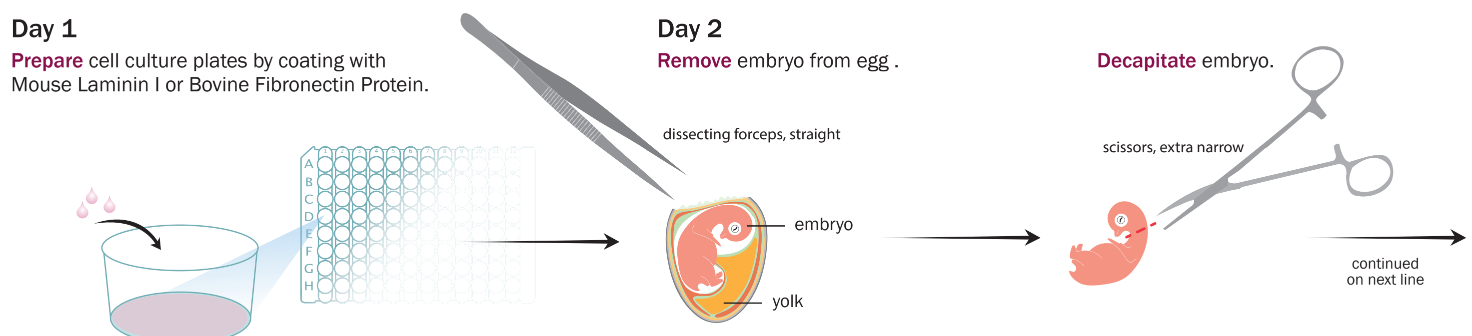
Exchanging Media in DRG Neuron Cultures

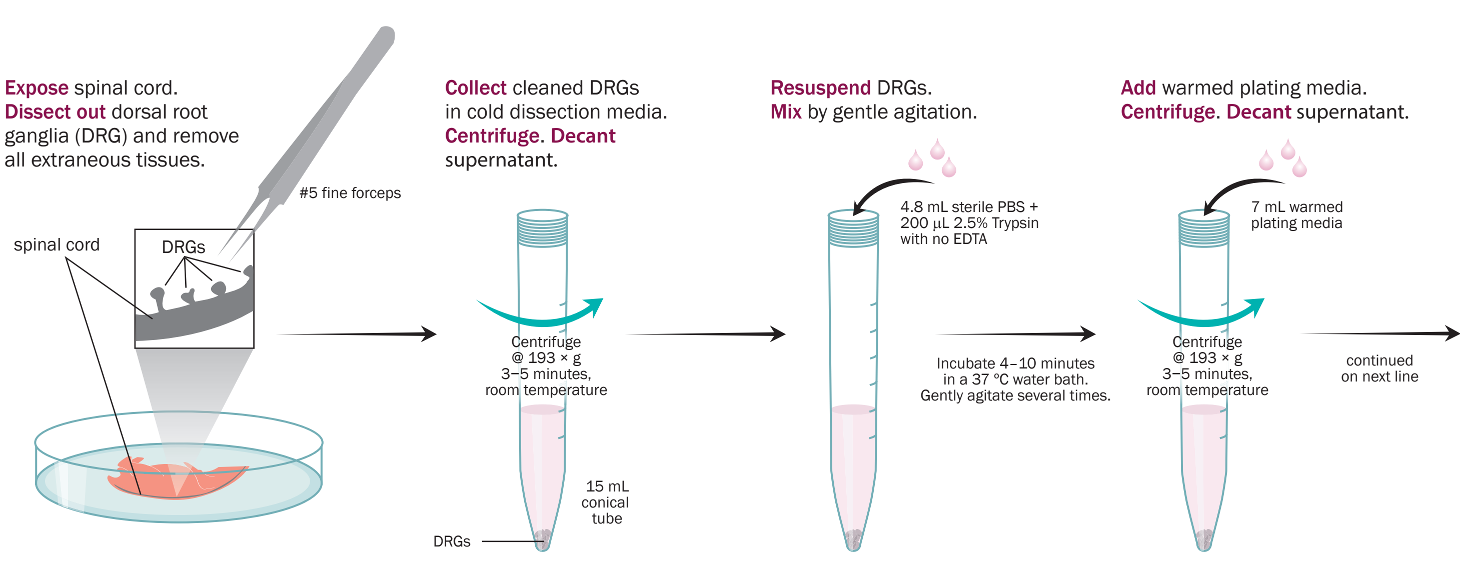
1. Warm an appropriate amount of culture media in a 37 °C, 5% CO_2 humidified incubator.
2. Gently remove half the volume of media (i.e. 50 µL) from each well of the cell culture plate. Gently add 50 µL of new, warmed culture media to each well of the cell culture plate.

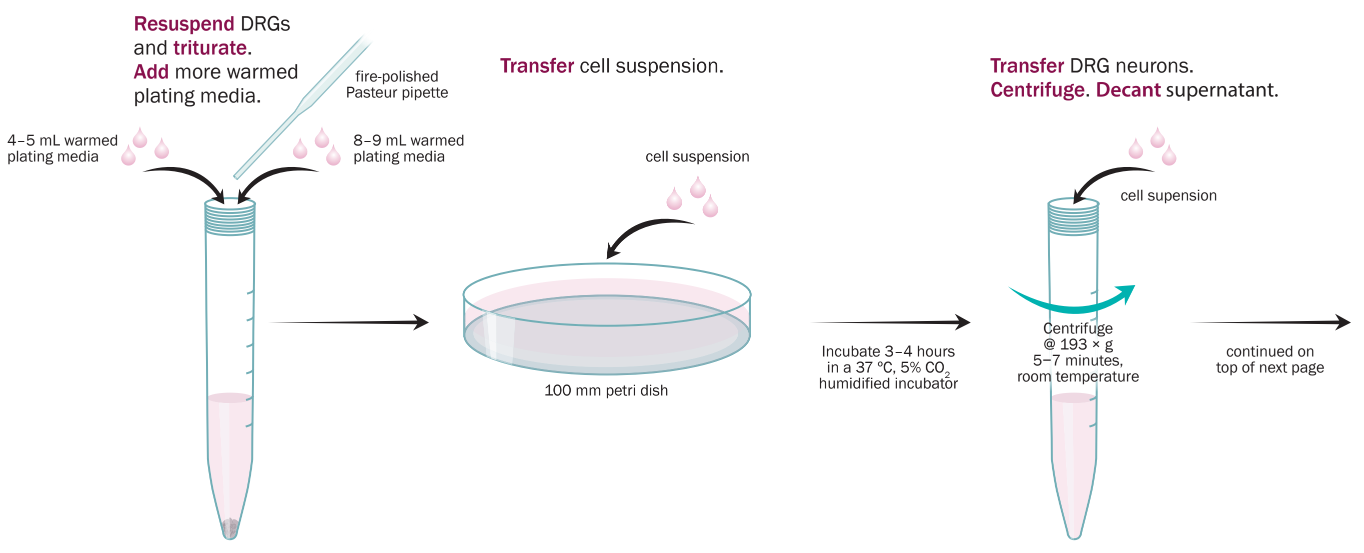
Note: Do not remove all the media from the wells of the cell culture plate as this will stress the DRG neurons.

3. Exchange the culture media every 3–4 days.

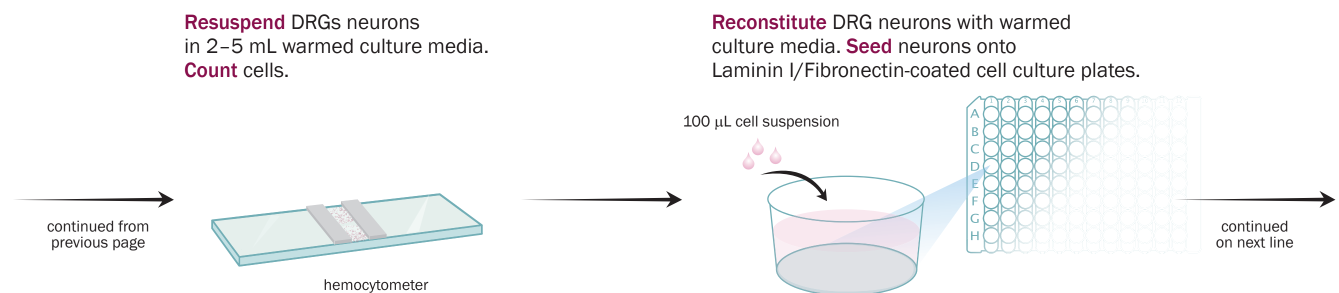
Protocol for Culturing Embryonic Chick Dorsal Root Ganglion Neurons



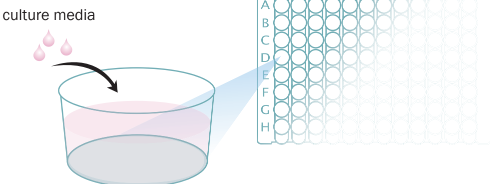




Protocol for Culturing Embryonic Chick Dorsal Root Ganglion Neurons, continued



Culture DRG neurons for desired amount of time. **Exchange** media every 3–4 days.



Protocol for Culturing Rat Cortical Neurons

Cortical neural cell cultures are an important model system for studying neuronal development and function, neurotoxicity screening, drug discovery, and mechanisms of neurological diseases. Proper development and survival of neurons, requires specific growth factors, signaling molecules, peptides, and vitamins.[1,2] This protocol provides step-by-step instructions for dissecting and culturing cortical neurons from both embryonic (E17–E18) and postnatal (P1–P2) rat pups.

Please read the protocol in its entirety before starting.

Note: In order to yield a healthy neuron population, brain tissue from P1–P2 rat pups needs to be enzymatically digested before trituration. Extra reagents and steps are required.

Note: Aseptic techniques should be used in this protocol to ensure there is no bacterial, fungal, or mycoplasma contamination. The initial dissection and collection of the cortical tissue can be completed outside of a laminar flow cell culture hood. However, preparation of reagents and cell culture plates, and all steps following tissue harvest, should be conducted within a hood. Likewise, all reagents and materials used should be sterile.

Supplies Required

Reagents

- Antibiotic-antimycotic (100x, ThermoFisher Scientific, Catalog # 15240062), or equivalent
- Cultrex® Mouse Laminin I (R&D Systems, Catalog # 3400-010-01)
- Cultrex® Poly-D-Lysine (R&D Systems, Catalog # 3439-100-01)
- Deionized or distilled H_2O, sterile (dH_2O)
- L-glutamine solution (200 mM, Irvine Scientific, Catalog # 9317), or equivalent
- NeuroXVivo™ Rat Cortical Neuron Culture Kit (R&D Systems, Catalog # CDK011)
- PBS, sterile (1x): 0.137 M NaCl, 0.05 M NaH_2PO_4, pH 7.4
- Trypan blue (0.4%)

Additional Reagents for P1–P2 Rat Pups

- DNase I (Worthington Biochemical Corp., Catalog # LK003170), or equivalent
- EBSS, Ca^{2+}, Mg^{2+}, phenol red (ThermoFisher Scientific, Catalog # 24010043)
- Ovomucoid protease inhibitor with BSA (Worthington Biochemical Corp., Catalog # LK003182), or equivalent
- Papain (Worthington Biochemical Corp., Catalog # LK003176), or equivalent

Materials

- 15 mL conical centrifuge tube
- Cell culture plates, sterile
- E17–E18 timed pregnant rat or P1–P2 rat pups
- Ice
- Parafilm®
- Pasteur pipette, glass, fire-polished, sterile
- Petri dishes, 60 × 15 mm
- Petri dishes, 100 × 20 mm
- Pipette tips

Note: Locate your desired seeding densities for your cortical neuron culture in Table 1 (pg. 30) to determine the size of the cell culture plate that should be used.

Equipment

- 37 °C, 5% CO_2 humidified incubator
- 37 °C water bath
- Autoclave
- Centrifuge
- Dissection microscope
- Dissecting tools
    - Dissecting forceps, curved (e.g. Gillies)
    - Fine forceps, #5, straight
    - Forceps, #7, curved
    - Surgical scissors, small
    - Surgical scissors, large
    - Vannas-Tübingen spring scissors, straight
- Hemocytometer
- Inverted microscope
- Laminar flow cell culture hood
- Pipettes

Reagent Preparation

Note: Prepare all solutions in a laminar flow cell culture hood.

Recombinant Human BDNF (1000x)

1. Add 560 µL of Reconstitution Buffer 1 to the vial of Recombinant Human BDNF

Recombinant Human IGF-I (1000x)

1. Add 600 µL of Reconstitution Buffer 1 to the vial of Recombinant Human IGF-I

Complete Cortical Neuron Culture Media

1. 1x N21-MAX Media Supplement,
   1x Recombinant Human BDNF,
   1x Recombinant Human IGF-I,
   1x antibiotic-antimycotic,
   0.5 mM L-glutamine in
   Neuronal Base Media

Note: Media is stable for up to 1 month at 2–8 °C after adding all the growth supplements.

Procedure

Coating and Preparation of Cell Culture Plates

Note: Preparation of the culture plates should be done in a laminar flow cell culture hood.

1. Dilute the Cultrex® Poly-D-Lysine solution with sterile dH_2O to a final concentration of 50 µg/mL.
2. Cover the wells of the culture plates with 50 µg/mL Cultrex® Poly-D-Lysine solution (e.g., 50 µL/well for 96-well plate). Tilt the plates to ensure even coating of the well surface.
3. Incubate plates for 1 hour in a 37 °C, 5% CO_2 humidified incubator.
4. Aspirate the poly-D-lysine solution. Wash the wells three times with sterile dH_2O. Aspirate the wells to completely remove all liquids.
5. Wrap plates with parafilm® to seal. Store plates at 2–8 °C for up to 2 weeks.

Note: Start the remaining steps the day before collection of the rat cortical tissue.

6. Dilute the Cultrex® Mouse Laminin I solution with sterile PBS to a final concentration of 10 µg/mL.
7. Cover the wells of the poly-D-lysine-coated plates with 10 µg/mL Cultrex® Mouse Laminin I (e.g., 50 µL/well for 96-well plate). Tilt the plates to ensure even coating of the well surface.
8. Incubate the plates overnight at 2–8 °C.

9. Aspirate the Laminin I solution from the wells prior to adding the cells. Wash the wells two times with sterile dH_2O. Aspirate the wells to remove all liquids.

Note: Alternatively, cortical neurons can be cultured on poly-L-Lysine coated, open µ-Slides (chambered #1.5 polymer coverslips; ibidi, Catalog # 80824).

Dissection of Rat Cortical Tissue

Note: Autoclave dissection tools to sterilize.

1. Warm an appropriate amount Neuronal Base Media and Complete Cortical Neuron Culture Media in a 37 °C water bath. Place sterile PBS on ice.

Note: If using P1–P2 rat pups, skip to step 4.

2. Asphyxiate the pregnant rat with CO_2. Recover embryos via cesarean section using the large surgical scissors and curved dissecting forceps. Place the embryos in a 100 × 20 mm petri dish containing cold PBS. Keep the dish on ice.
3. Remove the embryos from their individual placenta sacs and wash with cold PBS.
4. Place cleaned embryos in a new 100 × 20 mm petri dish containing cold PBS. Decapitate each embryo at the head/neck junction using the small surgical scissors. Decapitate P1–P2 rat pups with the small surgical scissors.
5. Place the heads in a new 100 × 20 mm petri dish containing cold PBS.
6. Stabilize the dissociated head using the #7 curved forceps and #5 fine forceps, Moving caudal to rostral, cut through the skull with the small surgical scissors.

Note: Keep cuts shallow to avoid damaging the brain tissue.

7. Peel back the two halves of the separated skull.
8. Remove the whole brain from the head cavity using the #7 curved forceps and place in a 60 × 15 mm petri dish containing cold PBS. Keep the dish on ice. Repeat steps 4–8 for the remaining heads.
9. Put a brain in a new 60 × 15 mm petri dish containing cold PBS. Under a dissecting microscope, cut the brain with the Vannas-Tübingen spring scissors, following the median longitudinal fissure, to separate the hemispheres. Cut off and discard any brain stem tissue.
10. With the #5 fine forceps, peel off the meninges that cover each hemisphere. Open up the brain to reveal the mid-sagittal side.
11. Locate the hippocampus, which is the darker, c-shaped region, and remove it using the Vannas-Tübingen spring scissors. Place the remaining cortical tissue in a new 60 × 15 mm petri dish containing cold PBS. Keep the dish on ice. Repeat steps 9–11 for the remaining brains.
12. Using the Vannas-Tübingen spring scissors, cut the isolated cortical tissue into smaller pieces (~2 mm^2).

Dissociation and Culture of Rat Cortical Neurons

Note: From this point forward, any opening of tubes/plates that contain any tissue, cells, media, or reagents should be done in a laminar flow cell culture hood.

Note: If using embryonic cortical tissue, start at step 1. If using P1–P2 tissue, start at step 3.

For embryonic cortical tissue:

1. Transfer the tissue pieces to a 15 mL conical tube. Add 5 mL of Neuronal Base Media. Gently triturate the tissue with the fire-polished Pasteur pipette until the solution is homogenous (~10–15 times).
2. Skip to step 6.

For postnatal cortical tissue:

3. In a 15 mL conical tube, mix 20 U/mL Papain and 100 U/mL DNase I in 5 mL of EBSS. Warm the solution in a 37 °C, 5% CO_2 humidified incubator for 10 minutes.
4. Transfer the tissue to the 15 mL conical tube containing the warmed enzyme solution. Incubate for 20–30 minutes in a 37 °C, 5% CO_2 humidified incubator.
5. Gently triturate the tissue with the fire-polished Pasteur pipette until the solution is homogenous (~10–15 times).
6. Centrifuge at 200 × g for 5 minutes at room temperature. Decant the solution.
7. Resuspend the cells in...

   For embryonic cortical tissue:
   10 mL of Neuronal Base Media.

   For postnatal cortical tissue:
   5 mL of EBSS containing 1 µg/mL of Ovomucoid protease inhibitor with BSA.
8. Centrifuge at 200 × g for 4–6 minutes at room temperature. Decant the solution.
9. Wash the cells twice with 10 mL of Neuronal Base Media. Centrifuge at 200 × g for 5 minutes at room temperature. Decant the media.
10. Resuspend the cells in warmed Complete Cortical Neuron Culture Media (~10 mL). Mix 10 µL of the cell suspension with 10 µL of 0.4% Trypan blue. Count the live cells.
11. Dilute the cell suspension to the desired seeding density (see Table 1, pg. 30) with warmed Complete Cortical Neuron Culture Media. Plate the neurons on the prepared culture plates.
12. Keep cultured cortical neurons in a 37 °C, 5% CO_2 humidified incubator until use.

Exchanging Media in Cortical Neuron Cultures

Note: Healthy cultures can be maintained for up to 4 weeks.

1. Warm an appropriate amount of Complete Cortical Neuron Culture Media in a 37 °C, 5% CO_2 humidified incubator.
2. Remove half the volume of media from each well of the culture plate (e.g., remove 50 µL from each well of a 96-well plate). Gently add an equal amount of new, warmed Completed Cortical Neuron Culture Media to each well.

Note: Do not remove all the media from the wells of the plate as this will stress the neurons.

3. Exchange the culture media every 3–4 days.

References

1. Catapano, L.A. *et al.* (2001) J. Neurosci. **21**:8863.
2. Martin, D.L. (1992) Glia **5**:81.

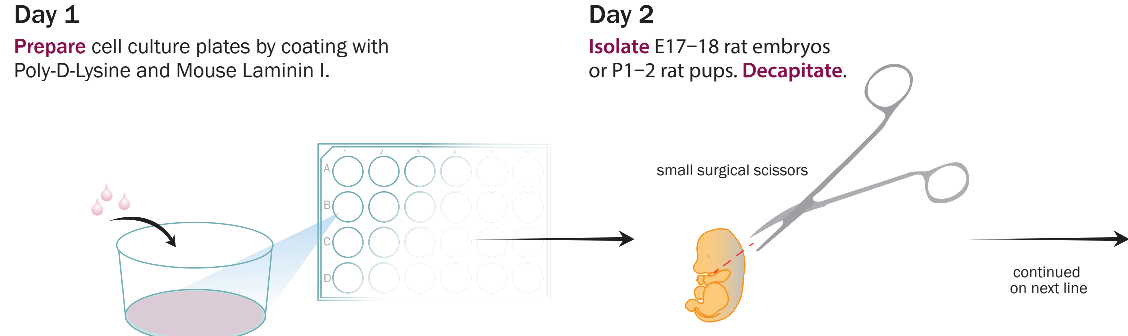
Protocol for Culturing Rat Cortical Neurons

Day 1

Prepare cell culture plates by coating with Poly-D-Lysine and Mouse Laminin I.

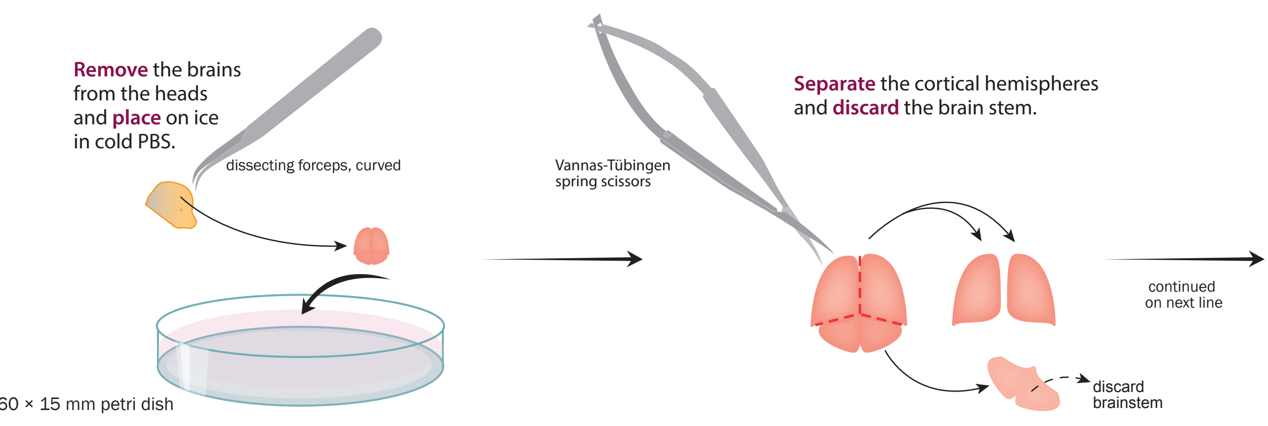
Day 2

Isolate E17–18 rat embryos or P1–2 rat pups. **Decapitate**.



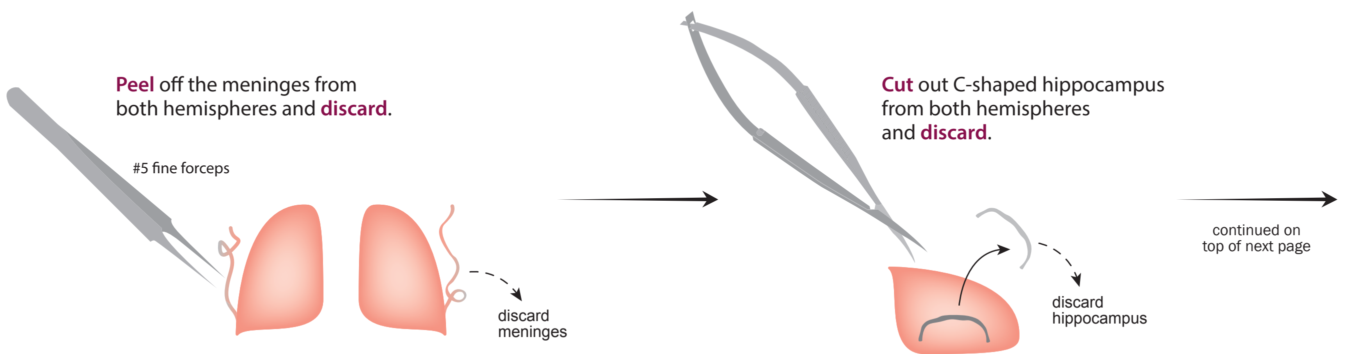
Remove the brains from the heads and **place** on ice in cold PBS.

Separate the cortical hemispheres and **discard** the brain stem.

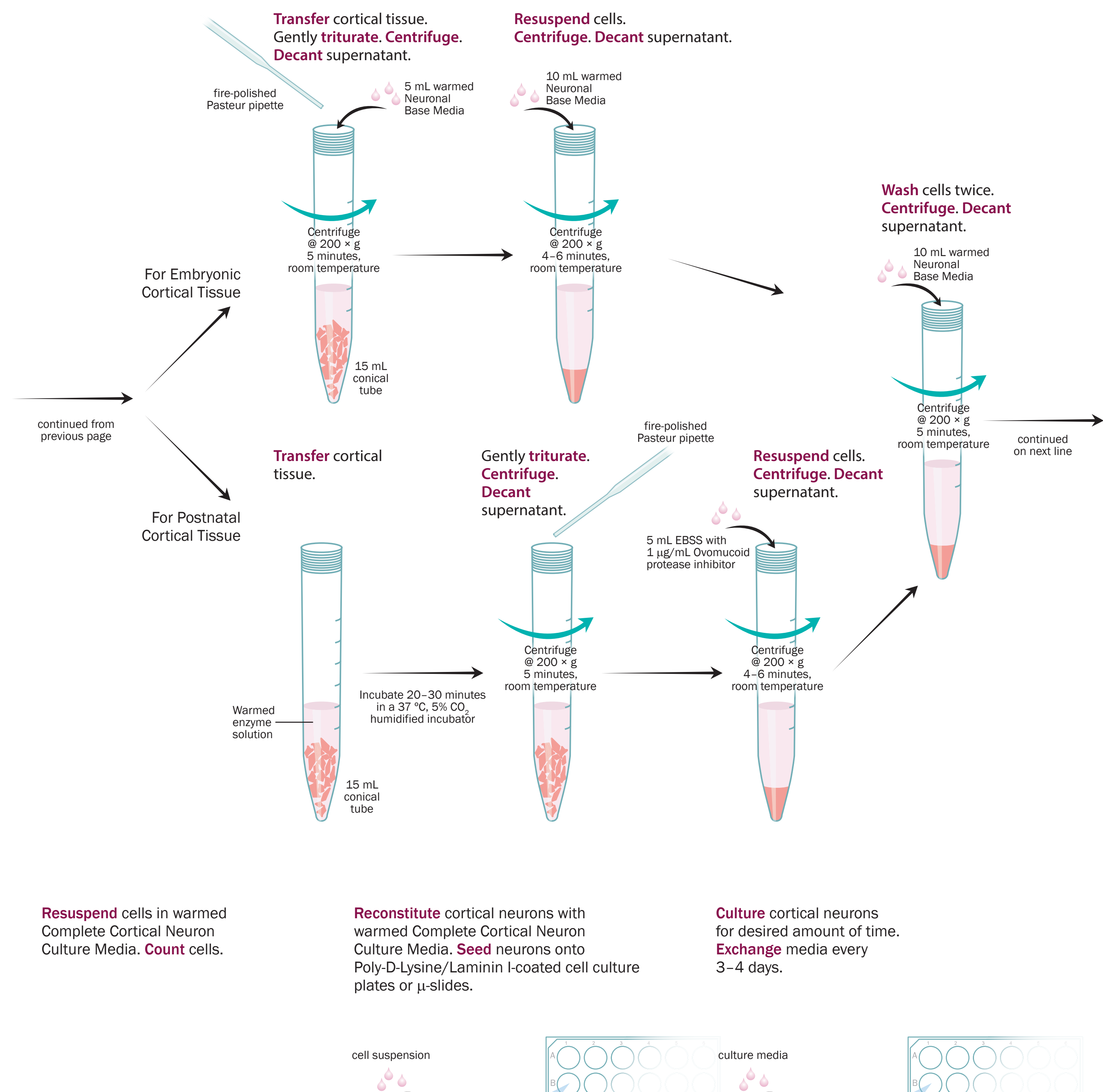


Peel off the meninges from both hemispheres and **discard**.

Cut out C-shaped hippocampus from both hemispheres and **discard**.



Protocol for Culturing Rat Cortical Neurons, continued



Protocol for Culturing Rat Hippocampal Neurons

Hippocampal neural cell cultures are a commonly used model system for not only investigating the physiological properties of learning and memory, but the cellular mechanisms of neurobiology in general. Hippocampal cell cultures contain relatively few interneurons, and these interneurons are morphologically distinguishable from pyramidal neurons, the major cell type in the hippocampus[1]. Another benefit of hippocampal cell cultures is that they form fully developed dendrites that are covered with spines and make substantial synaptic connections. This protocol provides step-by-step instructions for dissecting and culturing hippocampal neurons from both embryonic (E17–E18) and postnatal (P1–P2) rat pups.

Please read the protocol in its entirety before starting.

Note: In order to yield a healthy neuron population, brain tissue from P1–P2 rat pups needs to be enzymatically digested before trituration. Extra reagents and steps are required.

Note: Aseptic techniques should be used in this protocol to ensure there is no bacterial, fungal, or mycoplasma contamination. The initial dissection and collection of the hippocampi can be completed outside of a laminar flow cell culture hood. However, preparation of reagents and cell culture plates, and all steps following tissue harvest, should be conducted within a hood. Likewise, all reagents and materials used should be sterile.

Supplies Required

Reagents

- Antibiotic-antimycotic (100x, ThermoFisher Scientific, Catalog # 15240062), or equivalent
- Cultrex® Mouse Laminin I (R&D Systems, Catalog # 3400-010-01)
- Cultrex® Poly-D-Lysine (R&D Systems, Catalog # 3439-100-01)
- Deionized or distilled H_2O, sterile (dH_2O)
- DME medium, high glucose, no L-glutamine (Irvine Scientific, Catalog # 9024) or Neurobasal® medium (ThermoFisher Scientific, Catalog # 21103049), or equivalent
- L-glutamine solution (200 mM, Irvine Scientific, Catalog # 9317), or equivalent
- N21-MAX Media Supplement (50x, R&D Systems, Catalog # AR008)
- PBS, sterile (1x): 0.137 M NaCl, 0.05 M NaH_2PO_4, pH 7.4
- Trypan blue (0.4%)

Additional Reagents for P1–P2 Rat Pups:

- DNase I (Worthington Biochemical Corp., Catalog # LK003170), or equivalent
- EBSS, Ca^{2+}, Mg^{2+}, phenol red (ThermoFisher Scientific, Catalog # 24010043)
- Ovomucoid protease inhibitor with BSA (Worthington Biochemical Corp., Catalog # LK003182), or equivalent
- Papain (Worthington Biochemical Corp., Catalog # LK003176), or equivalent

Optional Reagents:

- Recombinant Human IGF-I Protein (R&D Systems, Catalog # 291-G1)
- Recombinant Human/Mouse/Rat/Canine/Equine BDNF Protein (R&D Systems, Catalog # 248-BD)

Materials

- 15 mL conical centrifuge tube
- Cell culture plates
- E17–E18 timed pregnant rat or P1–P2 rat pups
- Ice
- Parafilm®
- Pasteur pipette, glass, fire-polished, sterile
- Petri dishes, 60 × 15 mm
- Petri dishes, 100 × 20 mm
- Pipette tips

Note: Locate your desired seeding densities for your hippocampal cell culture in Table 1 (pg. 30) to determine the size of the cell culture plate that should be used.

Equipment

- 37 °C, 5% CO_2 humidified incubator
- 37 °C water bath
- Autoclave
- Centrifuge
- Dissecting microscope
- Dissection tools
  - Dissecting forceps, curved (e.g. Gillies)
  - Fine forceps, #5, straight
  - Forceps, #7, curved
  - Surgical scissors, small
  - Surgical scissors, large
  - Vannas-Tübingen spring scissors, straight
- Hemocytometer
- Inverted microscope
- Laminar flow cell culture hood
- Pipettes

Reagent Preparation

Note: Prepare all solutions in a laminar flow cell culture hood.

Culture Media

1. 1x N21-MAX Media Supplement, 1x antibiotic-antimycotic, 0.5 mM L-glutamine in DME (or Neurobasal®) medium

Note: Recombinant Human/Mouse/Rat/Canine/Equine BDNF Protein and Recombinant Human IGF-I Protein can be added to enhance the hippocampal cell culture.

Procedure

Coating and Preparation of Cell Culture Plates

Note: Preparation of the culture plates should be done in a laminar flow cell culture hood.

1. Dilute the Cultrex® Poly-D-Lysine solution with sterile dH_2O to a final concentration of 50 µg/mL.
2. Cover the wells of the culture plates with 50 µg/mL Cultrex® Poly-D-Lysine solution (e.g., 50 µL/well for 96-well plate). Tilt the plates to ensure even coating of the well surface.
3. Incubate plates for 1 hour in a 37 °C, 5% CO_2 humidified incubator.
4. Aspirate the poly-D-lysine solution. Wash the wells three times with sterile dH_2O. Aspirate the wells to remove all liquids.
5. Wrap plates with parafilm® to seal. Store plates at 2–8 °C for up to 2 weeks.

Note: Start these remaining steps the day before collection of the rat hippocampi.

6. Dilute the Cultrex® Mouse Laminin I solution with sterile PBS to a final concentration of 10 µg/mL.
7. Cover the wells of the poly-D-Lysine-coated plates with 10 µg/mL Cultrex® Mouse Laminin I (e.g., 50 µL/well for 96-well plate). Tilt the plates to ensure even coating of the well surface.
8. Incubate the plates overnight at 2–8 °C.

9. Aspirate the mouse Laminin I solution. Wash the wells two times with sterile dH_2O. Aspirate the wells to remove all liquids.

Note: Alternatively, hippocampal neurons can be cultured on poly-L-lysine coated, open µ-Slides (chambered #1.5 polymer coverslips; ibidi, Catalog # 80824).

Dissection of Rat Hippocampi

Note: Autoclave dissection tools to sterilize.

1. Warm an appropriate amount of DME (or Neurobasal®) medium and culture media in a 37 °C water bath. Place sterile PBS on ice.

Note: If using P1–P2 rat pups, skip to step 4.

2. Asphyxiate the pregnant rat with CO_2. Recover embryos via cesarean section using the large surgical scissors and curved dissecting forceps. Place the embryos in a 100 × 20 mm petri dish containing cold PBS. Keep the dish on ice.
3. Remove the embryos from their individual placenta sacs and wash with cold PBS.
4. Place cleaned embryos in a new 100 × 20 mm petri dish containing cold PBS. Decapitate each embryo at the head/neck junction using the small surgical scissors. Decapitate P1–P2 rat pups with the small surgical scissors.
5. Place the heads in a new 60 × 15 mm petri dish containing cold PBS.
6. Stabilize the dissociated head using the #7 curved forceps and #5 fine forceps. Moving caudal to rostral, cut through the skull with the small surgical scissors.

Note: Keep cuts shallow to avoid damaging the brain tissue.

7. Peel back the two halves of the separated skull.
8. Remove the whole brain from the head cavity using the #7 curved forceps and place in a 60 × 15 mm petri dish containing cold PBS. Keep the dish on ice. Repeat steps 4–8 for the remaining heads.
9. Put a brain in a new 60 × 15 mm petri dish containing cold PBS. Under a dissecting microscope, cut the brain with the Vannas-Tübingen spring scissors, following the median longitudinal fissure, to separate the hemispheres. Cut off and discard any brain stem tissue.
10. With the #5 fine forceps, peel off the meninges that cover each hemisphere. Open up the brain to reveal the mid-sagittal side.
11. Locate the hippocampus, which is the darker, c-shaped region, and remove it using the Vannas-Tübingen spring scissors. Place the hippocampal tissue in a new 60 × 15 mm petri dish containing cold PBS. Keep the dish on ice. Repeat steps 9–11 for the remaining brains.
12. Using the Vannas-Tübingen spring scissors, cut the isolated hippocampi into smaller pieces (~2 mm²).

Dissociation and Culture of Rat Hippocampal Neurons

Note: From this point forward, the opening of tubes/plates that contain any tissue, cells, media, or reagents should be done in a laminar flow cell culture hood.

Note: If using embryonic hippocampal tissue, start at step 1. If using P1–P2 tissue, start at step 3.

For embryonic hippocampi:

1. Transfer the tissue pieces to a 15 mL conical tube. Add 5 mL of DME (or Neurobasal®) medium. Gently triturate the tissue with the fire-polished Pasteur pipette until the solution is homogenous (~10–15 times).
2. Skip to step 6.

For postnatal hippocampi:

3. In a 15 mL conical tube, mix 20 U/mL Papain and 100 U/mL DNase I in 5 mL of EBSS. Warm the solution in a 37 °C, 5% CO_2 humidified incubator for 10 minutes.
4. Transfer the tissue to the 15 mL conical tube containing the warmed enzyme solution. Incubate for 20–30 minutes in a 37 °C, 5% CO_2 humidified incubator.
5. Gently triturate the tissue with the fire-polished Pasteur pipette until the solution is homogenous (~10–15 times).
6. Centrifuge at 200 × g for 5 minutes at room temperature. Decant the solution.
7. Resuspend the cells in...

For embryonic hippocampi:
10 mL of DME (or Neurobasal®) medium.

For postnatal hippocampi:
5 mL of EBSS containing 1 µg/mL of Ovomucoid protease inhibitor with BSA.

8. Centrifuge at 200 × g for 4–6 minutes at room temperature. Decant the solution.
9. Wash the cells twice with 10 mL of DME (or Neurobasal®) medium. Centrifuge at 200 × g for 5 minutes at room temperature. Decant the media.
10. Resuspend the cells in warmed culture media (~ 10 mL). Mix 10 µL of the cell suspension with 10 µL of 0.4% Trypan blue. Count the live cells.
11. Dilute the cell suspension to the desired seeding density (see Table 1, pg. 30) with warmed culture media. Plate the neurons on the prepared culture plates.
12. Keep cultured neurons in a 37 °C, 5% CO_2 humidified incubator until use.

Exchanging Media in Hippocampal Neuron Cultures

Note: Healthy cultures can be maintained for up to 4 weeks.

1. Warm an appropriate amount of culture media in a 37 °C, 5% CO_2 humidified incubator.
2. Remove half the volume of media from each well of the culture plate (e.g., remove 50 µL from each well of a 96-well plate). Gently add an equal amount of new, warmed, culture media to each well.

Note: Do not remove all the media from the wells of the plate as this will stress the neurons.

3. Exchange the culture media every 3–4 days.

References

1. Kaech, S. and G. Banker (2006) Nat. Protoc. **1**:2406.

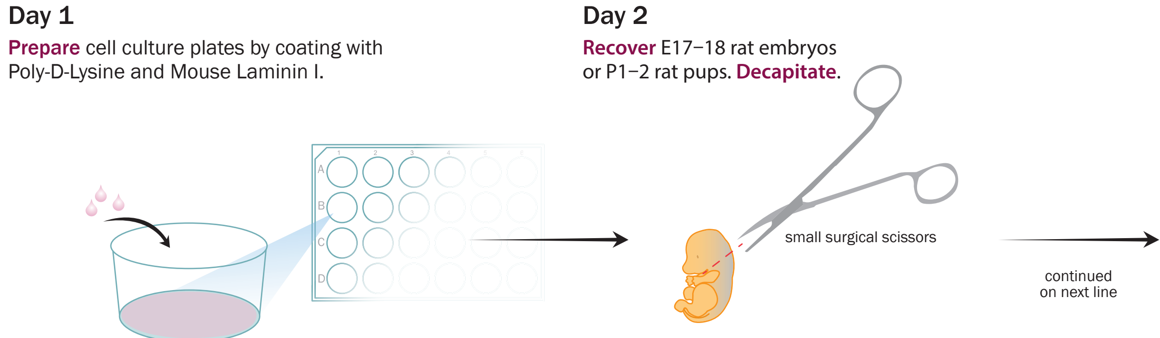
Protocol for Culturing Rat Hippocampal Neurons

Day 1

Prepare cell culture plates by coating with Poly-D-Lysine and Mouse Laminin I.

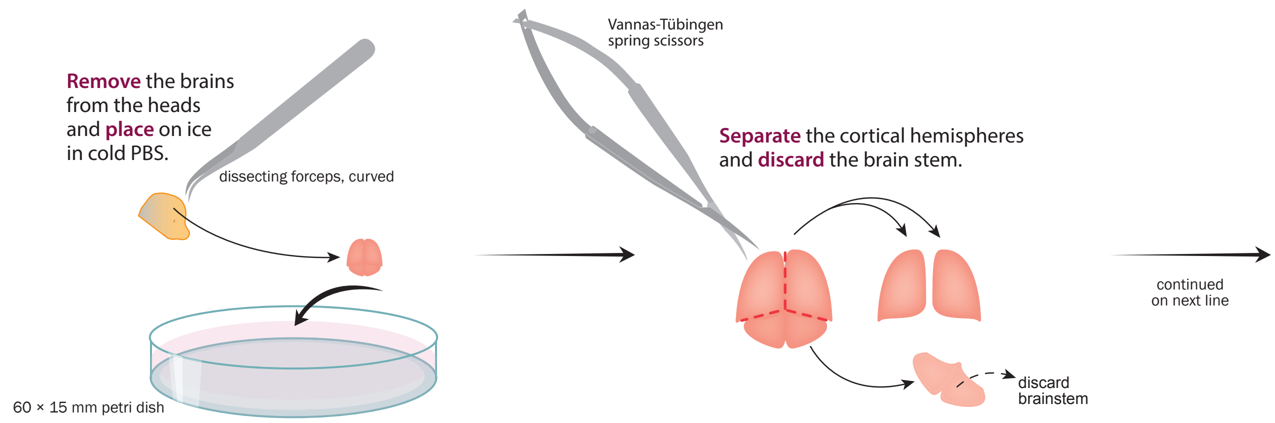
Day 2

Recover E17–18 rat embryos or P1–2 rat pups. **Decapitate**.



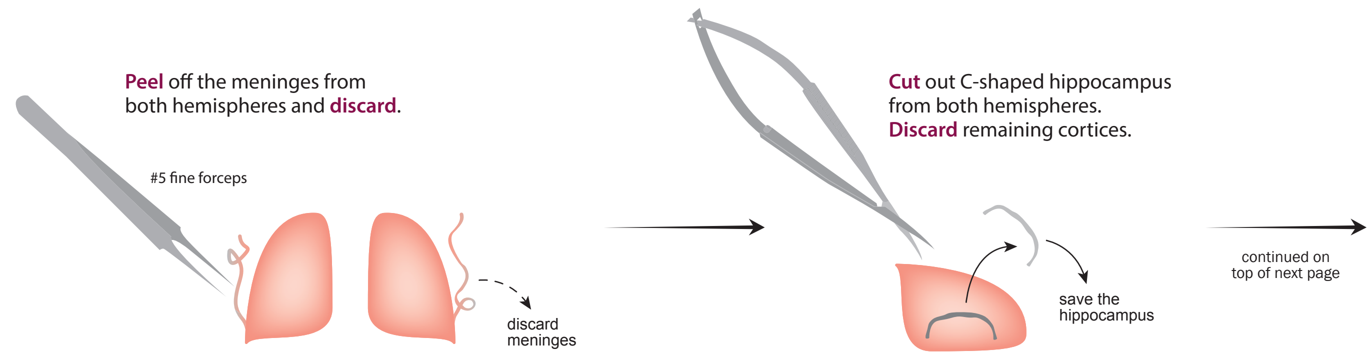
Remove the brains from the heads and **place** on ice in cold PBS.

Separate the cortical hemispheres and **discard** the brain stem.



Peel off the meninges from both hemispheres and **discard**.

Cut out C-shaped hippocampus from both hemispheres. **Discard** remaining cortices.



Protocol for Culturing Rat Hippocampal Neurons, continued

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For Embryonic Hippocampal Tissue

fire-polished Pasteur pipette

Transfer hippocampal tissue. Gently **triturate**. **Centrifuge**. **Decant** supernatant.

5 mL warmed Media

Centrifuge @ 200 × g 5 minutes, room temperature

15 mL conical tube

Resuspend cells. **Centrifuge**. **Decant** supernatant.

10 mL warmed Media

Centrifuge @ 200 × g 4–6 minutes, room temperature

For Postnatal Hippocampal Tissue

Transfer hippocampal tissue.

Warmed enzyme solution

15 mL conical tube

Incubate 20–30 minutes in a 37 °C, 5% CO_2 humidified incubator

Gently **triturate**. **Centrifuge**. **Decant** supernatant.

fire-polished Pasteur pipette

Centrifuge @ 200 × g 5 minutes, room temperature

Resuspend cells. **Centrifuge**. **Decant** supernatant.

5 mL EBSS with 1 µg/mL Ovomucoid protease inhibitor

Centrifuge @ 200 × g 4–6 minutes, room temperature

Wash cells twice. **Centrifuge**. **Decant** supernatant.

10 mL warmed media

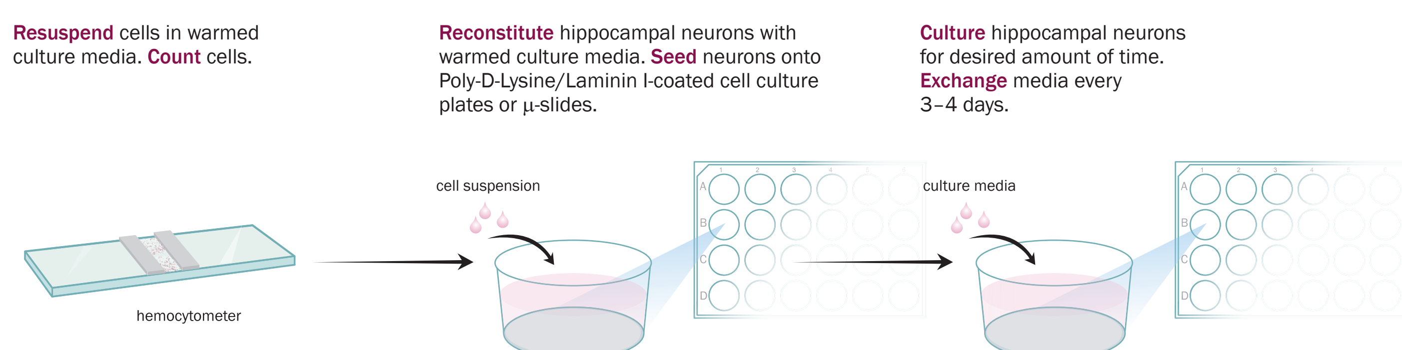
Centrifuge @ 200 × g 5 minutes, room temperature

continued on next line

Resuspend cells in warmed culture media. **Count** cells.

Reconstitute hippocampal neurons with warmed culture media. **Seed** neurons onto Poly-D-Lysine/Laminin I-coated cell culture plates or µ-slides.

Culture hippocampal neurons for desired amount of time. **Exchange** media every 3–4 days.



Protocol for Culturing Mouse Cortical Stem Cells: Expansion Using the Neurosphere System

Ex vivo expanded neural stem cells serve as excellent tools for researchers studying neural development and neurological disorders. Ready-to-use primary cortical stem cells, isolated from E14.5 CD-1 mice, can be grown in a monolayer or as neurospheres. The neurosphere culture system described here is useful for investigating processes involved in neural stem cell proliferation and differentiation. Neurospheres are cultured in defined, serum-free media enabling researchers to interrogate stem cell growth and differentiation by modulating cues in the extrinsic environment. Neural stem cells grown using this culture system retain the capacity for multilineage differentiation into astrocytes, neurons, and oligodendrocytes.

Please read the protocol in its entirety before starting.

Note: Aseptic techniques should be used in this protocol to ensure there is no bacterial, fungal, or mycoplasma contamination. All steps should be conducted within a laminar flow cell culture hood, and all reagents and materials used should be sterile.

Caution: The mouse cortical stem cells used in this protocol contain trace amounts of human Transferrin and DMSO, and the media used in this protocol contains trace amounts of Transferrin. The Transferrin was tested at the donor level using an FDA licensed method and found to be non-reactive for anti-HIV 1/2 and Hepatitis B surface antigen. Because no testing can offer complete assurance of freedom from infectious agents, these reagents should be handled as if capable of transmitting infection.

Supplies Required

Reagents

- Acetic acid
- Bovine Serum Albumin (BSA), media grade, very low endotoxin (EMD Millipore, Catalog # 810683), or equivalent
- Deionized H_2O (DI H_2O)
- DMEM/F-12, powder (ThermoFisher Scientific, Catalog # 12500062), or equivalent
- Glucose
- Glutamine
- Mouse Cortical Stem Cells (R&D Systems, Catalog # NSC002)
- N-2 MAX Media Supplement (100x, R&D Systems, Catalog # AR009)
- $NaHCO_3$
- PBS, 1x-Dulbecco's, no Ca^{2+}, no Mg^{2+}, no phenol red (Irvine Scientific, Catalog # 9240), or equivalent
- Recombinant Human EGF Protein (R&D Systems, Catalog # 236-EG)
- Recombinant Human FGF basic Protein (R&D Systems, Catalog # 233-FB or 4114-TC)
- Trypan blue (0.4%)

Optional Reagents

- Penicillin-streptomycin (100x, ThermoFisher Scientific, Catalog # 15140148), or equivalent

Materials

- 0.2 µm, 500 mL, sterile filter unit
- 15 mL conical centrifuge tubes, sterile
- 50 mL conical centrifuge tubes, sterile
- Cell culture plates (6-well), sterile (Corning™ # 3516), or equivalent
- Pipette tips
- Serological pipettes

Equipment

- 37 °C, 5% CO_2 humidified incubator
- 37 °C water bath
- Centrifuge
- Hemocytometer
- Laminar flow cell culture hood
- Inverted microscope
- Pipette pump
- Pipettes

Reagent Preparation

Note: Sterile technique is required when handling the reagents so to maintain aseptic conditions

Completed Neural Stem Cell (NSC) Base Media

1. Mix the following components with DI H_2O to make 500 mL of Completed NSC Base Media.

Reagent	Amount
DMEM/F-12	6 g
Glucose	0.775 g
Glutamine	0.0365 g
$NaHCO_3$	0.845 g
N-2 MAX Media Supplement	5 mL

2. Adjust the pH to 7.2 ± 0.2. Sterile filter the solution using a 0.2 µm filter unit.

Note: Penicillin-streptomycin can be added to the media at a final concentration of 1x. Completed NSC Base Media can be stored for up to 2 weeks at 2–8 °C in the dark.

FGF basic Stock Solution (20 µg/mL)

1. Add sterile 0.1% BSA in PBS to the Human FGF basic vial to make a 20 µg/mL stock solution.

EGF Stock Solution (20 µg/mL)

1. Add sterile 0.1% BSA in 10 mM acetic acid to the Human EGF vial to make a 20 µg/mL stock solution.

Note: Aliquot and store protein stock solutions at ≤ –20 °C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.

Procedure

Thawing Cryopreserved Cells

Note: Review the following protocol in detail before thawing the cells.

1. Add the FGF basic and EGF stock solutions to 30 mL of Completed NSC Base Media to a final concentration of 20 ng/mL. Warm the media in a 37 °C water bath.
2. Add 20 mL of warmed Completed NSC Base Media with mitogens to a sterile 50 mL conical tube. Reserve the remaining 10 mL of warmed Completed NSC Base Media for step 5.

3. Remove the cryovial containing frozen Mouse Cortical Stem Cells from the liquid nitrogen. Using a 2 mL pipette, immediately add 1 mL of fresh, warmed media to the vial. Gently pipette the media up and down. As the cells begin to thaw, transfer the thawed portion into the warmed media in the 50 mL conical tube. Repeat this process with the warmed media until all of the cells have thawed.

Note: Most of the frozen cells will be at the bottom of the cryovial.

4. Centrifuge the cells at 200 × g for 5 minutes at room temperature.
5. Carefully aspirate off 95% of the supernatant. Resuspend the cells in 10 mL of warmed Completed NSC Base Media with mitogens by gently pipetting.

Note: Rapid resuspension of frozen cells in warmed media during thawing is critical. Allowing cells to thaw slowly in the DMSO will dramatically reduce viability. Around 90% cell viability is expected from the freshly thawed cells when the appropriate thawing procedure is followed.

6. Mix 10 µL of the cell suspension with 10 µL of 0.4% Trypan blue. Count the live cells. Seed cells at a density according to the appropriate expansion protocol.

Cell Expansion

Note: Use serological pipettes to transfer and remove solutions.

1. Warm an appropriate amount of Completed NSC Base media. If needed, add EGF and FGF basic stock solutions to the media at a final concentration of 20 ng/mL.
2. Seed approximately 2×10^5 Mouse Cortical Stem Cells in 5 mL of warmed Completed NSC Base Media supplemented with 20 ng/mL EGF and 20 ng/mL FGF basic per well in a 6-well cell culture plate.
3. Keep cells in a 37 °C, 5% CO_2 humidified incubator.
4. Add fresh EGF (20 ng/mL) and FGF basic (20 ng/mL) each day to the media. Every fourth day, replace the media according to the number of neurospheres present.

For 50 neurospheres or more:

a. Transfer the media containing the neurospheres to a 15 mL conical tube.

b. Centrifuge at 100 × g for 5 minutes at room temperature. Decant the media.

c. Gently resuspend the pellet using a small quantity of fresh, warmed Completed NSC Base Media containing 20 ng/mL EGF and 20 ng/mL FGF basic.

d. Add the neurosphere suspension to one well of a 6-well cell culture plate that contains 5 mL of fresh, warmed Completed NSC Base Media with 20 ng/mL EGF and 20 ng/mL FGF basic.

For less than 50 neurospheres:

a. Transfer the neurospheres directly into one well of a 6-well cell culture plate that contains 2.5 mL of fresh, warmed Completed NSC Base Media with 20 ng/mL EGF and 20 ng/mL FGF basic.

Note: Do not discard the conditioned media. When there are fewer neurospheres, conditioned media is required. Only half of the media is replaced with fresh, warmed Completed NSC Base Media containing 20 ng/mL EGF and 20 ng/mL FGF basic.

b. Add 2.5 mL of the conditioned media to the well.

5. Pass the cells at 5–7 days, or when the neurospheres have a dark clump inside, or ruffling on the outside of the neurosphere, according to the procedure described below.

Passaging Cells

1. Warm an appropriate amount of Completed NSC Base Media. If needed, add EGF and FGF basic stock solutions to the media at a final concentration of 20 ng/mL.
2. Transfer the media containing the floating neurospheres to a 15 mL conical tube. Do not dislodge attached neurospheres for passage.
3. Centrifuge for 5 minutes at 100 × g at room temperature.
4. Partially dissociate the pelleted neurospheres by pipetting up and down 20 times, being careful not to create bubbles in the suspension.

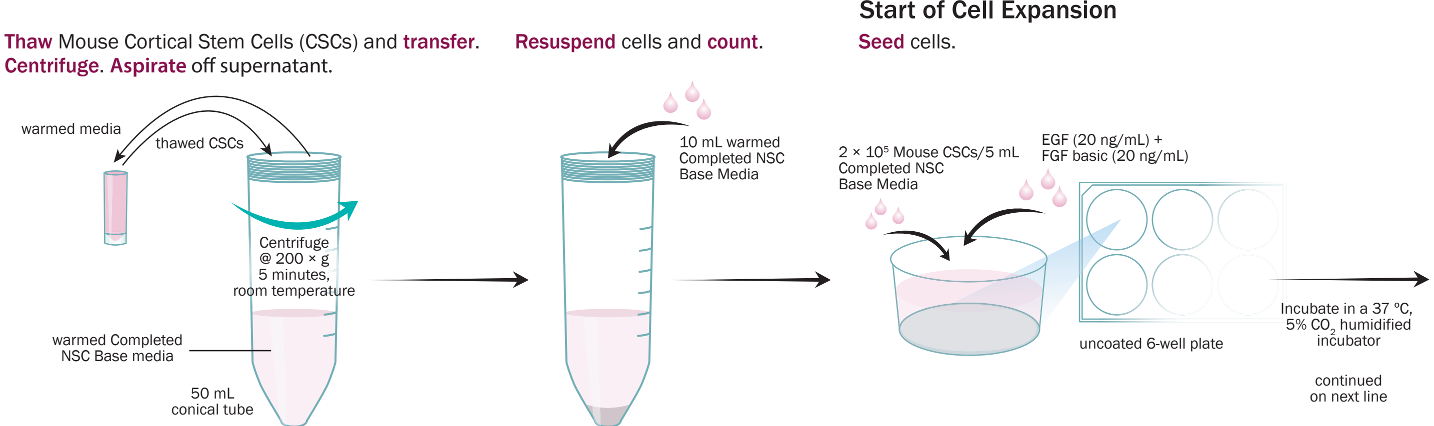
Note: For optimal dissociation of the neurospheres, it is recommended that a P200 pipette be used.

5. At the initial passages 1 and 2, add 5 mL of fresh, warmed Completed NSC Base Media with mitogens to the partially dissociated neurospheres. Transfer the final neurosphere suspension into one well of a 6-well cell culture plate. Repeat from step 3 in the Cell Expansion section (see above).
6. After passage 2, add 10 mL of fresh, warmed Completed NSC Base Media with mitogens to the partially dissociated neurospheres. Split the final neurosphere suspension into two wells of a 6-well cell culture plate (5 mL of neurosphere suspension/well). Repeat from step 3 in the Cell Expansion section (see above).

References

1. Johe, K.K. *et al.* (1996) Genes Dev. **10**:3129.
2. Kim, J.H. *et al.* (2003) Methods Enzymol. **365**:303.
3. Tropepe, V. *et al.* (1999) Dev. Biol. **208**:166.
4. T.J. Kilpatrick and P.F. Bartlett (1993) Neuron **10**:255.

Protocol for Culturing Mouse Cortical Stem Cells: Expansion Using the Neurosphere System



Feed cells.

Daily: Add EGF (20 ng/mL) + FGF Basic (20 ng/mL)

Every 4th day: Completely change media

Less than 50 Neurospheres

Transfer individual neurospheres to a new plate.

Add media.

2.5 mL Conditioned Media

2.5 mL Completed NSC Base Media + EGF (20 ng/mL) and FGF (20 ng/mL)

More than 50 Neurospheres

Transfer media containing neurospheres. **Centrifuge**. **Decant** supernatant.

Centrifuge @ 100 × g 5 minutes, room temperature

15 mL conical tube

Resuspend the neurospheres and **transfer** to one well of a culture plate.

5 mL Completed NSC Base Media + EGF (20 ng/mL) and FGF (20 ng/mL)

Cell suspension

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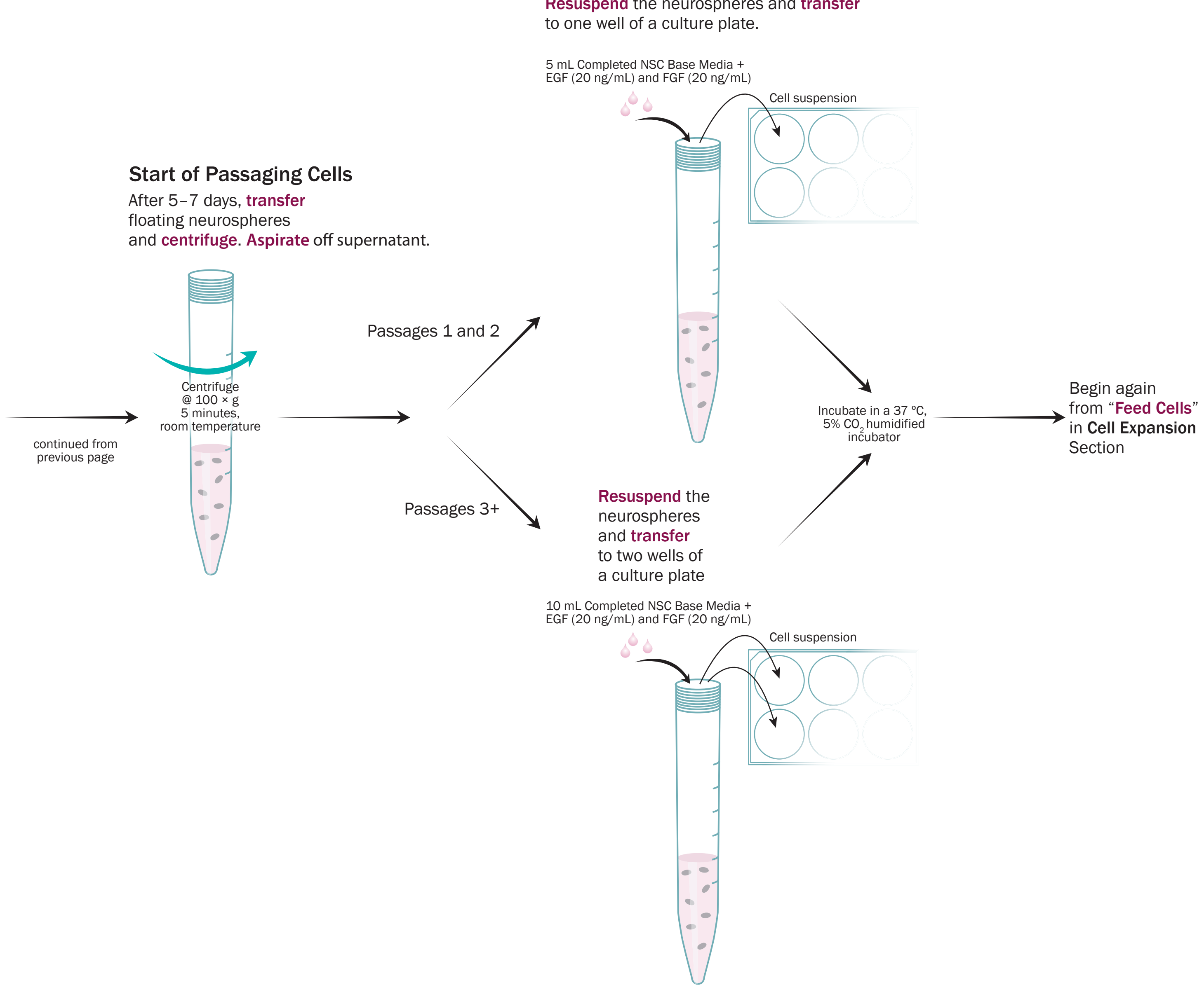
Protocol for Culturing Mouse Cortical Stem Cells: Expansion Using the Neurosphere System, continued



Protocol for Culturing Rat Cortical Stem Cells: Expansion Using the Neurosphere System

Ex vivo expanded neural stem cells serve as excellent tools for researchers studying neural development and neurological disorders. Ready-to-use primary cortical stem cells, isolated from E14.5 Sprague-Dawley rats, can be grown in a monolayer or as neurospheres. The neurosphere culture system described here is useful for investigating processes involved in neural stem cell proliferation and differentiation. Neurospheres are cultured in defined, serum-free media enabling researchers to interrogate stem cell growth and differentiation by modulating cues in the extrinsic environment. Neural stem cells grown using this culture system retain the capacity for multilineage differentiation into astrocytes, neurons, and oligodendrocytes.

Please read the protocol in its entirety before starting.

Note: Aseptic techniques should be used in this protocol to ensure there is no bacterial, fungal, or mycoplasma contamination. All steps should be conducted within a laminar flow cell culture hood, and all reagents and materials used should be sterile.

Caution: The rat cortical stem cells used in this protocol contain trace amounts of human Transferrin and DMSO, and the media used in this protocol contains trace amounts of Transferrin. The Transferrin was tested at the donor level using an FDA licensed method and found to be non-reactive for anti-HIV 1/2 and Hepatitis B surface antigen. Because no testing can offer complete assurance of freedom from infectious agents, these reagents should be handled as if capable of transmitting infection.

Supplies Required

Reagents

- Acetic acid
- Bovine Serum Albumin (BSA), media grade, very low endotoxin (EMD Millipore, Catalog # 810683), or equivalent
- Deionized H_2O (DI H_2O)
- DMEM/F-12, powder (ThermoFisher Scientific, Catalog # 12500062), or equivalent
- Glucose
- Glutamine
- Rat Cortical Stem Cells (R&D Systems, Catalog # NSC001)
- N-2 MAX Media Supplement (100x, R&D Systems, Catalog # AR009)
- $NaHCO_3$
- PBS, 1x-Dulbecco's, no Ca^{2+}, no Mg^{2+}, no phenol red (Irvine Scientific, Catalog # 9240), or equivalent
- Recombinant Human EGF Protein (R&D Systems, Catalog # 236-EG)
- Recombinant Human FGF basic Protein (R&D Systems, Catalog # 233-FB or 4114-TC)
- Trypan blue (0.4%)

Optional Reagents

- Penicillin-streptomycin (100x, ThermoFisher Scientific, Catalog # 15140148), or equivalent

Materials

- 0.2 µm, 500 mL, sterile filter unit
- 15 mL conical centrifuge tubes, sterile
- 50 mL conical centrifuge tubes, sterile
- Cell culture plates (6-well), sterile (Corning™ # 3516), or equivalent
- Pipette tips
- Serological pipettes

Equipment

- 37 °C, 5% CO_2 humidified incubator
- 37 °C water bath
- Centrifuge
- Hemocytometer
- Laminar flow cell culture hood
- Inverted microscope
- Pipette pump
- Pipettes

Reagent Preparation

Note: Sterile technique is required when handling the reagents so to maintain aseptic conditions

Completed Neural Stem Cell (NSC) Base Media

1. Mix the following components with DI H_2O to make 500 mL of Completed NSC Base Media.

Reagent	Amount
DMEM/F-12	6 g
Glucose	0.775 g
Glutamine	0.0365 g
NaHCO3	0.845 g
N-2 MAX Media Supplement	5 mL

2. Adjust the pH to 7.2 ± 0.2. Sterile filter the solution using a 0.2 µm filter unit.

Note: Penicillin-streptomycin can be added to the media at a final concentration of 1x. Completed NSC Base Media can be stored for up to 2 weeks at 2–8 °C in the dark.

FGF basic Stock Solution (20 µg/mL)

1. Add sterile 0.1% BSA in PBS to the Human FGF basic vial to make a 20 µg/mL stock solution.

EGF Stock Solution (20 µg/mL)

1. Add sterile 0.1% BSA in 10 mM acetic acid to the Human EGF vial to make a 20 µg/mL stock solution.

Note: Aliquot and store protein stock solutions at ≤ –20 °C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.

Procedure

Thawing Cryopreserved Cells

Note: Review the following protocol in detail before thawing the cells.

1. Add the FGF basic and EGF stock solutions to 30 mL of Completed NSC Base Media to a final concentration of 20 ng/mL. Warm the media in a 37 °C water bath.

2. Add 20 mL of warmed Completed NSC Base Media with mitogens to a sterile 50 mL conical tube. Reserve the remaining 10 mL of warmed Completed NSC Base Media for step 5.

3. Remove the cryovial containing frozen Rat Cortical Stem Cells from the liquid nitrogen. Using a 2 mL pipette, immediately add 1 mL of fresh, warmed media to the vial. Gently pipette the media up and down. As the cells begin to thaw, transfer the thawed portion into the warmed media in the 50 mL conical tube. Repeat this process with the warmed media until all of the cells have thawed.

Note: Most of the frozen cells will be at the bottom of the cryovial.

4. Centrifuge the cells at 200 × g for 5 minutes at room temperature.
5. Carefully aspirate off 95% of the supernatant. Resuspend the cells in 10 mL of warmed Completed NSC Base Media with mitogens by gently pipetting.

Note: Rapid resuspension of frozen cells in warmed media during thawing is critical. Allowing cells to thaw slowly in the DMSO will dramatically reduce viability. Around 90% cell viability is expected from the freshly thawed cells when the appropriate thawing procedure is followed.

6. Mix 10 µL of the cell suspension with 10 µL of 0.4% Trypan blue. Count the live cells. Seed cells at a density according to the appropriate expansion protocol.

Cell Expansion

Note: Use serological pipettes to transfer and remove solutions.

1. Warm an appropriate amount of Completed NSC Base media. If needed, add EGF and FGF basic stock solutions to the media at a final concentration of 20 ng/mL.
2. Seed approximately 1×10^5 Rat Cortical Stem Cells in 5 mL of warmed Completed NSC Base Media supplemented with 20 ng/mL EGF and 20 ng/mL FGF basic per well in a 6-well cell culture plate.
3. Keep cells in a 37 °C, 5% CO_2 humidified incubator.
4. Add fresh EGF (20 ng/mL) and FGF basic (20 ng/mL) each day to the media. Every fourth day, replace the media according to the number of neurospheres present.

For 50 neurospheres or more:

a. Transfer the media containing the neurospheres to a 15 mL conical tube.
b. Centrifuge at 100 × g for 5 minutes at room temperature. Decant the media.
c. Gently resuspend the pellet using a small quantity of fresh, warmed Completed NSC Base Media containing 20 ng/mL EGF and 20 ng/mL FGF basic.
d. Add the neurosphere suspension into one well of a 6-well cell culture plate that contains 5 mL of fresh, warmed Completed NSC Base Media with 20 ng/mL EGF and 20 ng/mL FGF basic.

For less than 50 neurospheres:

a. Transfer the neurospheres directly into one well of a 6-well cell culture plate that contains 2.5 mL of fresh, warmed Completed NSC Base Media containing 20 ng/mL EGF and 20 ng/mL FGF basic.

Note: Do not discard the conditioned media. When there are fewer neurospheres, conditioned media is required. Only half of the media is replaced with fresh, warmed Completed NSC Base Media containing 20 ng/mL EGF and 20 ng/mL FGF basic.

b. Add 2.5 mL of the conditioned media to the well.

5. Pass the cells at 5–7 days, or when the neurospheres have a dark clump inside, or ruffling on the outside of the neurosphere, according to the procedure described below.

Passaging Cells

1. Warm an appropriate amount of Completed NSC Base Media. If needed, add EGF and FGF basic stock solutions to the media at a final concentration of 20 ng/mL.
2. Transfer the media containing the floating neurospheres to a 15 mL conical tube. Do not dislodge attached neurospheres for passage.
3. Centrifuge for 5 minutes at 100 × g at room temperature.
4. Partially dissociate the pelleted neurospheres by pipetting up and down 20 times, being careful not to create bubbles in the suspension.

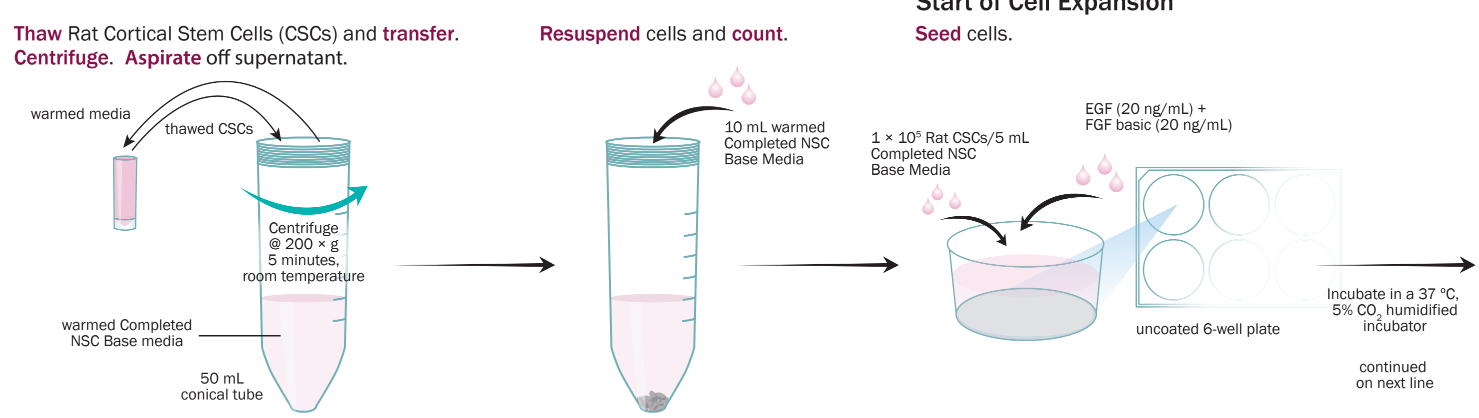
Note: For optimal dissociation of the neurospheres, it is recommended that a P200 pipette be used.

5. At the initial passages 1 and 2, add 5 mL of fresh, warmed Completed NSC Base Media with mitogens to the partially dissociated neurospheres. Transfer the final neurosphere suspension into one well of a 6-well cell culture plate. Repeat from step 3 in the Cell Expansion section (see above).
6. After passage 2, add 10 mL of fresh, warmed Completed NSC Base Media with mitogens to the partially dissociated neurospheres. Split the final neurosphere suspension into two wells of a 6-well cell culture plate (5 mL of neurosphere suspension/well). Repeat from step 3 in the Cell Expansion section (see above).

References

1. Johe, K.K. *et al.* (1996) Genes Dev. **10**:3129.
2. Kim, J.H. *et al.* (2003) Methods Enzymol. **365**:303.

Protocol for Culturing Rat Cortical Stem Cells: Expansion Using the Neurosphere System



Feed cells.

Daily: Add EGF (20 ng/mL) + FGF Basic (20 ng/mL)

Every 4th day: Completely change media

Less than 50 Neurospheres

Transfer individual neurospheres to a new plate.

Add media.

2.5 mL Conditioned Media

2.5 mL Completed NSC Base Media + EGF (20 ng/mL) and FGF (20 ng/mL)

More than 50 Neurospheres

Transfer media containing neurospheres. **Centrifuge.Decant** supernatant.

Centrifuge @ 100 × g 5 minutes, room temperature

15 mL conical tube

Resuspend the neurospheres and **transfer** to one well of a culture plate.

5 mL Completed NSC Base Media + EGF (20 ng/mL) and FGF (20 ng/mL)

Cell suspension

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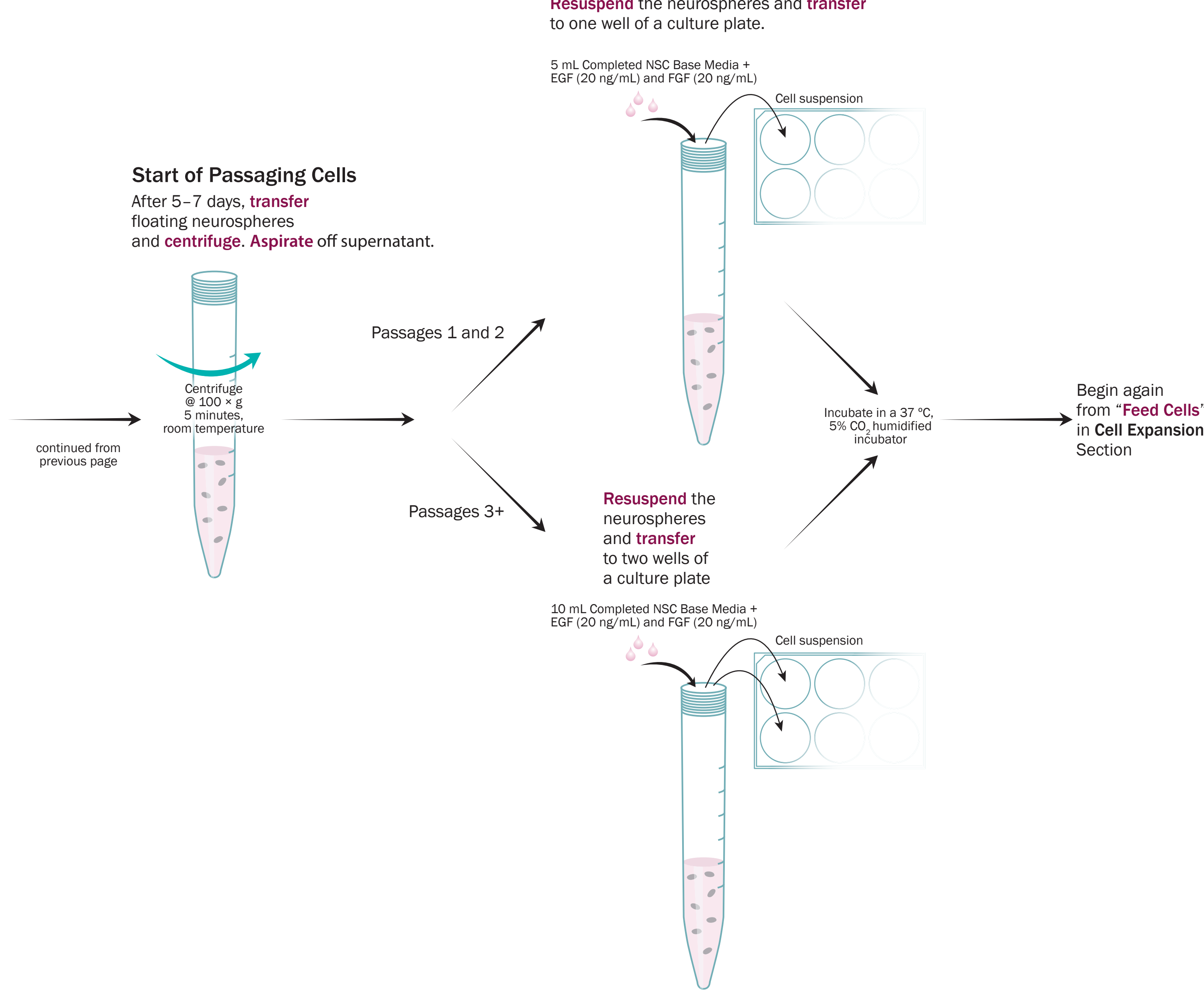
Protocol for Culturing Rat Cortical Stem Cells: Expansion Using the Neurosphere System, continued



Protocol for Culturing Rat Cortical Stem Cells: Expansion Using the Monolayer System

Ex vivo expanded neural stem cells serve as excellent tools for researchers studying neural development and neurological disorders. Ready-to-use primary cortical stem cells, isolated from E14.5 Sprague-Dawley rats can be grown in a monolayer, as described here, or as neurospheres. Monolayer cultures provide a more homogenous population of undifferentiated precursor cells and help to inhibit spontaneous differentiation. Additionally, cells grown in a monolayer can be directly monitored and investigated using methods such as functional assays and immunocytochemistry. Neural stem cells grown using this culture system retain the capacity for multilineage differentiation into astrocytes, neurons, and oligodendrocytes.

Please read the protocol in its entirety before starting.

Note: Aseptic techniques should be used in this protocol to ensure there is no bacterial, fungal, or mycoplasma contamination. All steps should be conducted within a laminar flow cell culture hood, and all reagents and materials used should be sterile.

Caution: The rat cortical stem cells used in this protocol contain trace amounts of human Transferrin and DMSO. The Transferrin was tested at the donor level using an FDA licensed method and found to be non-reactive for anti-HIV 1/2 and Hepatitis B surface antigen. Because no testing can offer complete assurance of freedom from infectious agents, these reagents should be handled as if capable of transmitting infection.

Supplies Required

Reagents

- Bovine Fibronectin Protein (R&D Systems, Catalog # 1030-FN)
- Bovine Serum Albumin (BSA), media grade, very low endotoxin (EMD Millipore, Catalog # 810683), or equivalent
- Deionized H_2O (dH_2O)
- DMEM/F-12, powder (ThermoFisher Scientific, Catalog # 12500062), or equivalent
- Glucose
- Glutamine
- HBSS, no Ca^{2+}, no Mg^{2+}, no phenol red (10x, ThermoFisher Scientific, Catalog # 14185052)
- HEPES sodium salt (Sigma-Aldrich, Catalog # H7006), or equivalent
- N-2 MAX Media Supplement (100x, R&D Systems, Catalog # AR009)
- $NaHCO_3$
- PBS, 1x-Dulbecco's, no Ca^{2+}, no Mg^{2+}, no phenol red, (Irvine Scientific, Catalog # 9240), or equivalent
- Poly-L-ornithine hydrobromide (Sigma-Aldrich, Catalog # P3655), or equivalent
- Rat Cortical Stem Cells (R&D Systems, Catalog # NSC001)
- Recombinant Human FGF basic Protein (R&D Systems, Catalog # 233-FB)
- Trypan blue (0.4%)

Optional Reagents

- Penicillin-streptomycin (100x, ThermoFisher Scientific, Catalog # 15140148), or equivalent

Materials

- 0.2 µm, 500 mL, sterile filter units
- 0.2 µm, 1000 mL, sterile filter units
- 10 cm tissue culture dishes, sterile
- 50 mL conical centrifuge tubes
- Plastic cell scraper, sterile
- Pipette tips

Equipment

- 37 °C, 5% CO_2 humidified incubator
- 37 °C water bath
- Centrifuge
- Hemocytometer
- Inverted microscope
- Pipettes

Reagent Preparation

Note: Sterile technique is required when handling the reagents so to maintain aseptic conditions

Completed Neural Stem Cell (NSC) Base Media

1. Mix the following components with DI H_2O to make 500 mL of Completed NSC Base Media.

Reagent	Amount
DMEM/F-12	6 g
Glucose	0.775 g
Glutamine	0.0365 g
NaHCO3	0.845 g
N-2 MAX Media Supplement	5 mL

2. Adjust the pH to 7.2 ± 0.2. Sterile filter the solution using a 500 mL, 0.2 µm filter unit.

Note: Penicillin-streptomycin can be added to the media at a final concentration of 1x. Completed NSC Base Media can be stored for up to 2 weeks at 2–8 °C in the dark.

Buffered HBSS (1x)

1. Add 100 mL of HBSS (10x) and 3.9 g HEPES to 900 mL of dH_2O.
2. Adjust the pH to 7.2 ± 0.2. Sterile filter the solution using a 1000 mL, 0.2 µm filter unit.

Note: Buffered HBSS can be stored at room temperature for up to 6 months.

FGF basic Stock Solution (20 µg/mL)

1. Add sterile 0.1% BSA to the Human FGF basic vial to make a 20 µg/mL stock solution.

Poly-L-Ornithine Stock Solution (15 mg/mL)

1. Add poly-L-ornithine hydrobromide to sterile PBS to make a 15 mg/mL stock solution.

Note: Aliquot and store the FGF basic and poly-L-ornithine stock solutions at ≤ –20 °C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.

Procedure

Culture Dish Preparation

1. Dilute the 15 mg/mL poly-L-ornithine solution with sterile PBS to a final concentration of 15 μg/mL.

Note: Prepare this solution fresh as needed.

2. Add 10 mL of the 15 μg/mL poly-L-ornithine solution to each 10 cm tissue culture dish. Incubate the dishes overnight in a 37 °C, 5% CO_2 humidified incubator.
3. Discard the poly-L-ornithine solution. Wash each tissue culture dish three times with 10 mL of sterile PBS. Add 10 mL of sterile PBS to each dish. Incubate the tissue culture dishes overnight in a 37 °C, 5% CO_2 humidified incubator.
4. Allow the vial of bovine Fibronectin protein to warm to room temperature without agitation. Add sterile PBS to the bovine Fibronectin protein to make a final concentration of 1 μg/mL. Gently invert the vial to mix.

Note: Prepare this solution fresh as needed.

5. Discard the PBS from each of the poly-L-ornithine-coated dishes. Wash each tissue culture dish once with 10 mL of sterile PBS.
6. Add 10 mL of the 1 μg/mL bovine Fibronectin solution to each tissue culture dish. Incubate the dishes for 3–30 hours in a 37 °C, 5% CO_2 humidified incubator.
7. Prior to use, discard the bovine Fibronectin solution and wash each tissue culture dish once with 10 mL of sterile PBS.

Thawing Cryopreserved Cells

Note: Review the following section in detail before thawing the cells.

1. Add the FGF basic stock solution to 30 mL of Completed NSC Base Media to a final concentration of 20 ng/mL. Warm the media in a 37 °C water bath.
2. Add 20 mL of warmed Completed NSC Base Media with FGF basic to a sterile 50 mL conical centrifuge tube. Reserve the remaining 10 mL of warmed Completed NSC Base Media for step 5.
3. Remove the cryovial containing frozen Rat Cortical Stem Cells from the liquid nitrogen. Using a 2 mL pipette, immediately add 1 mL of fresh, warmed media to the vial. Gently pipette the media up and down. As the cells begin to thaw, transfer the thawed portion into the warmed media in the 50 mL conical tube. Repeat this process with the warmed media until all of the cells have thawed.

Note: Most of the frozen cells will be at the bottom of the cryovial.

4. Mix 10 μL of the cell suspension with 10 μL of 0.4% Trypan blue and count the live cells.
5. Seed cells at a density according to the expansion protocol described below.

Cell Expansion

1. Seed 1–1.5 × 10^6 Rat Cortical Stem Cells in 10 mL of warmed Completed NSC Base Media supplemented with 20 ng/mL of FGF basic on a poly-L-ornithine/Fibronectin-coated tissue culture dish.
2. Incubate the tissue culture plates for 3 hours to overnight in a 37 °C, 5% CO_2 humidified incubator. After the cells become adherent, replace the media with fresh, warmed Completed NSC Base Media supplemented with 20 ng/mL FGF basic and return the plates to the 37 °C, 5% CO_2 humidified incubator.
3. After 24 hours, add 10 μL of the 20 μg/mL FGF basic stock solution to the culture.
4. Each day, supplement the media in the tissue culture plates with 10 μL of the 20 μg/mL FGF basic stock solution. Every second day, replace the media with fresh, warmed Completed NSC Base Media.
5. When the cultures reach 60–70% confluency (~ 4 days after initial plating), passage the cells according to the procedure described below.

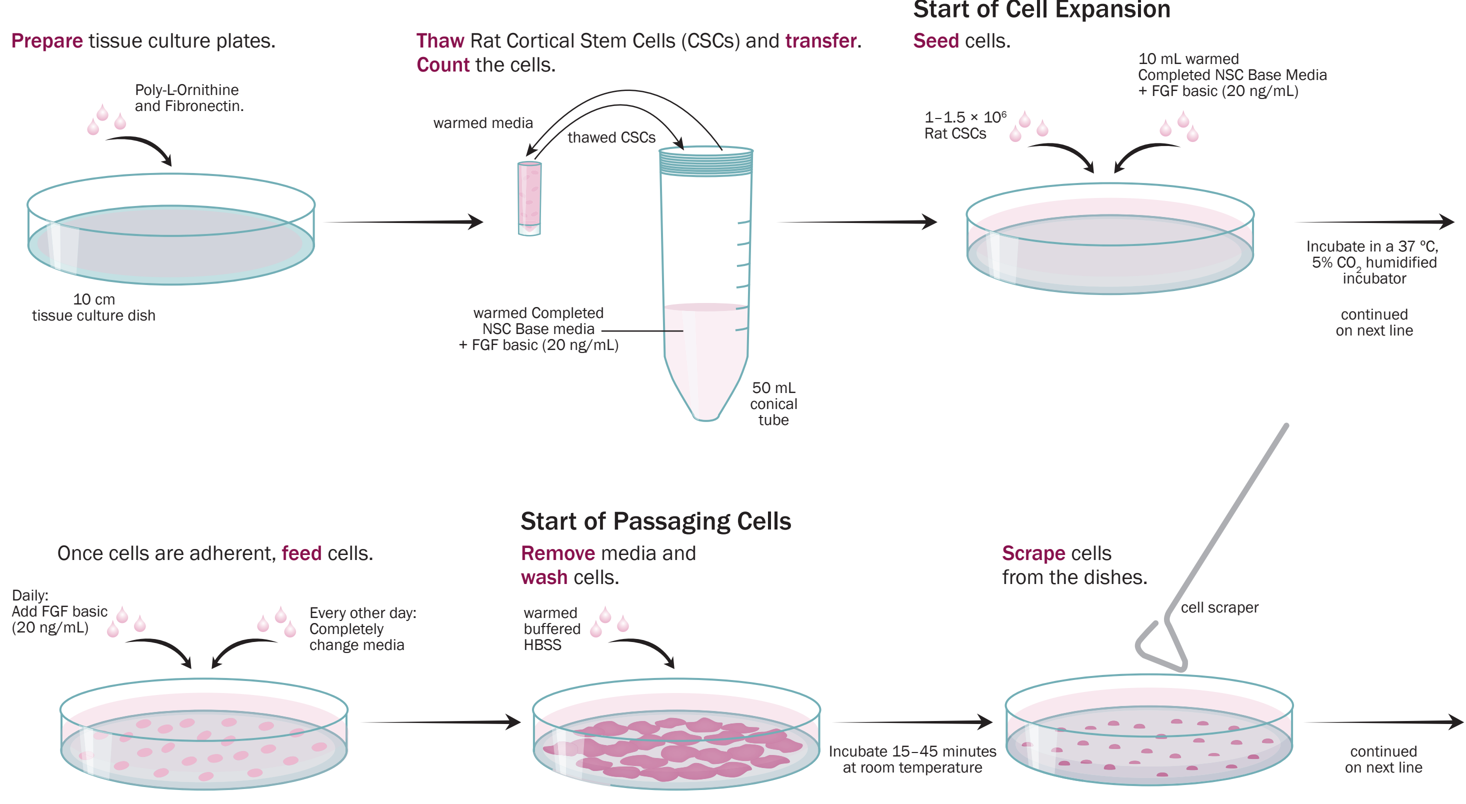
Passaging Cells

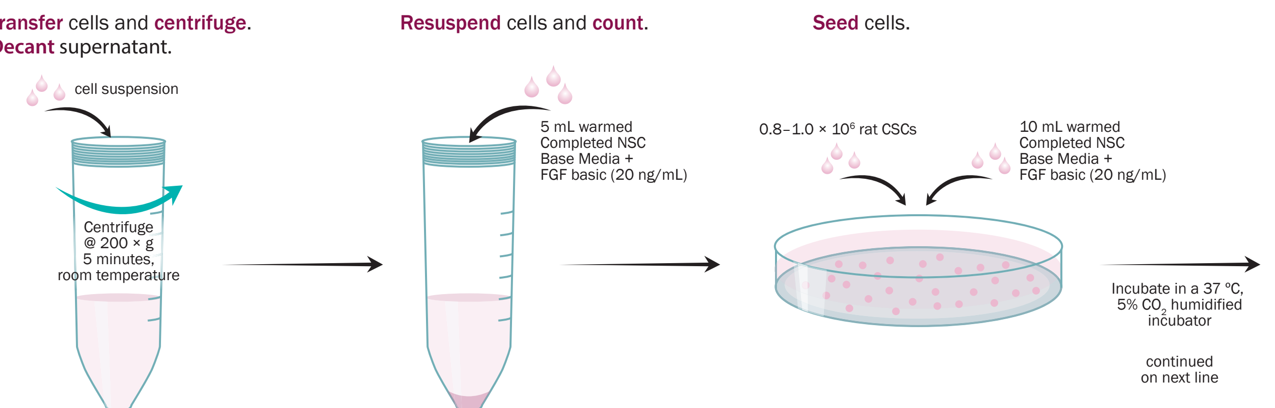
1. Warm the buffered HBSS (1x) and Completed NSC Base Media supplemented with 20 ng/mL FGF basic in a 37 °C water bath.
2. Remove the media from the cells. Wash the cells once with 10 mL of warmed buffered HBSS (1x).
3. Add 5 mL of warmed buffered HBSS (1x) to the tissue culture plates. Incubate the plates for 15–45 minutes at room temperature until the cells round up.

Note: Check the cultures frequently.

4. Scrape the cells from the tissue culture plates with a sterile, hard plastic cell scraper. Transfer the cells to a sterile 50 mL conical tube. Centrifuge at 200 × g for 5 minutes at room temperature. Decant the solution from the pelleted cells.
5. Add 5 mL of warmed Completed NSC Base Media supplemented with 20 ng/mL FGF basic to the pelleted cells. Using a 5 mL pipette, resuspend the cells by slowly pipetting up and down (~5 times).
6. Mix 10 μL of the cell suspension with 10 μL of 0.4% Trypan blue. Count the live cells.
7. Seed 0.8–1.0 × 10^6 viable cells on a poly-L-ornithine/Fibronectin-coated plate in 10 mL of warmed Completed NSC Base Media supplemented with 20 ng/mL FGF basic.
8. Incubate the cells in a 37 °C, 5% CO_2 humidified incubator. Repeat steps 4 and 5 in the Cell Expansion section (see above). Passage the cells after 3 days or when cells reach 70% confluency.

Protocol for Culturing Rat Cortical Stem Cells: Expansion Using the Monolayer System





Once cells are adherent, **feed** cells.

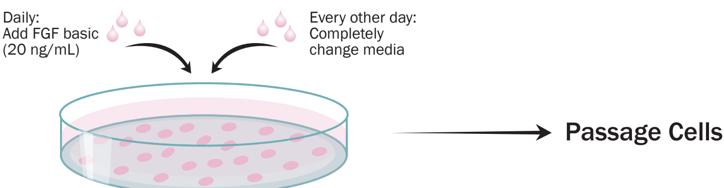
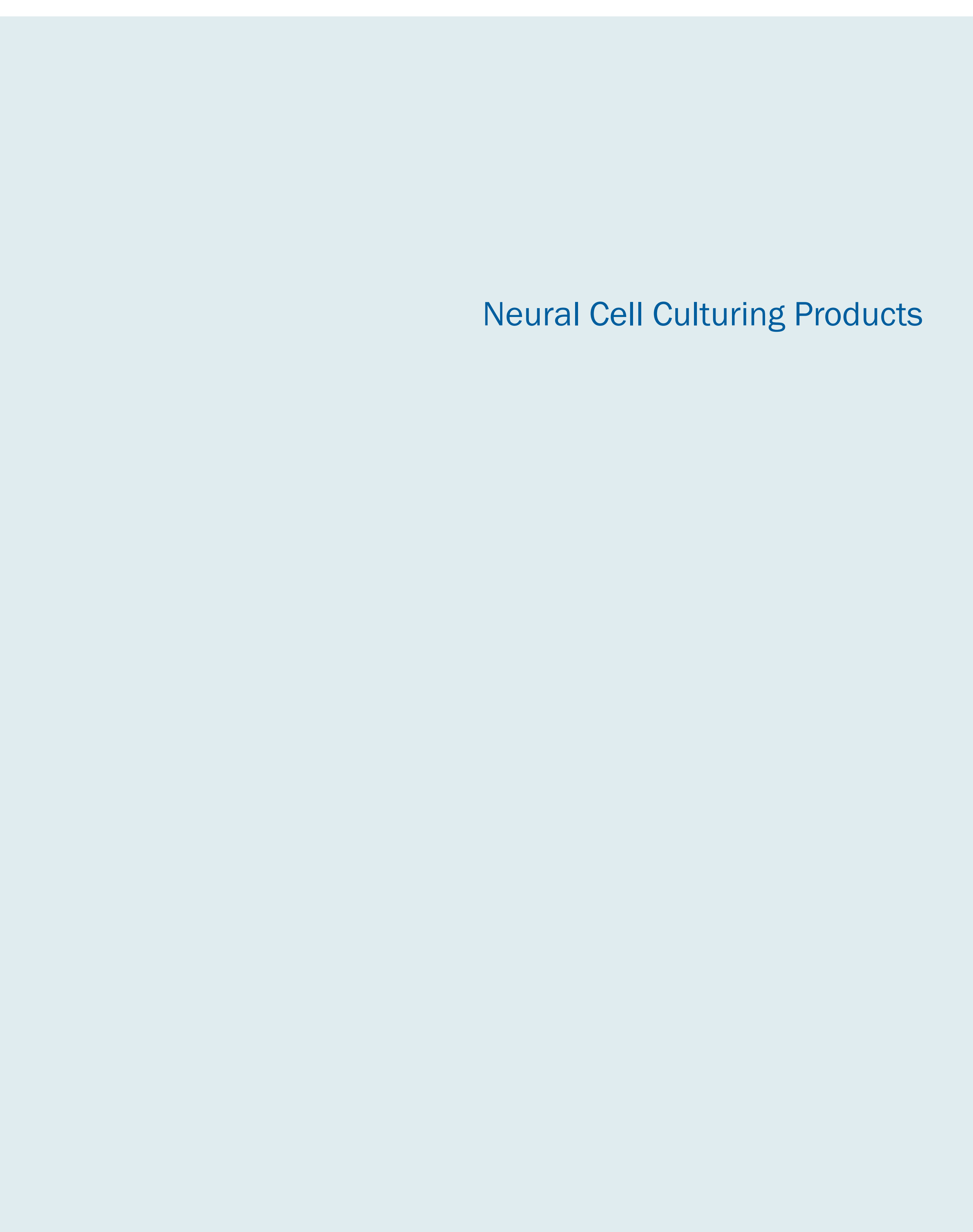


Table 1. Recommended Seeding Densities for Cortical/Hippocampal Neuron Cultures

Culture Dishes/ Chamber Slides	Media Volume/ Well (mL)	Total Number of Cells Required to Seed Each Well		
		Low Density (2.5×10^4 cells/cm^2)	Medium Density (5.0×10^4 cells/cm^2)	High Density (2.5×10^5 cells/cm^2)
6-well plate	2.0	2×10^5	5×10^5	2×10^6
12-well plate	0.8	1×10^5	2×10^5	1×10^6
24-well plate	0.5	5×10^4	1×10^5	5×10^5
48-well plate	0.3	2.5×10^4	5×10^4	2.5×10^5
96-well plate	0.1	5×10^3	2×10^4	5×10^4
4-well μ-slide	0.5	5×10^4	1×10^5	5×10^5
8-well μ-slide	0.3	2×10^4	4×10^4	2×10^5



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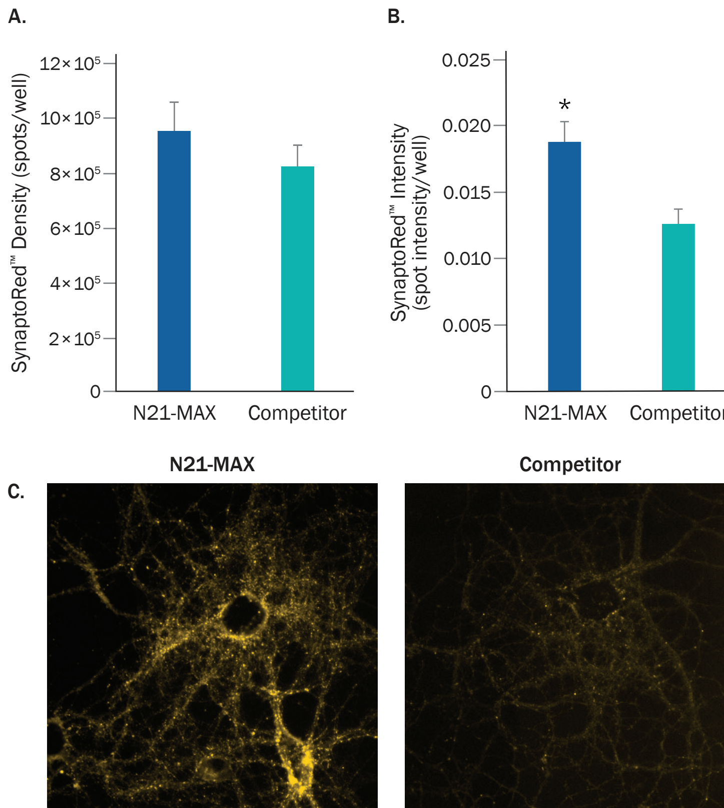
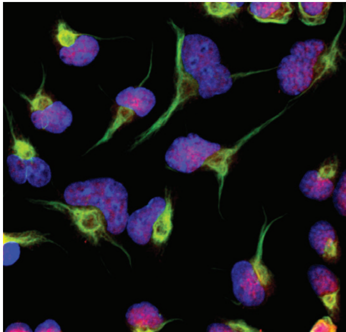
- **Culture with Confidence.**
  From cell culture substrates and media supplements to differentiation kits and imaging dyes, our tools for cell culture are designed to ensure that your *in vitro* and *ex vivo* experiments perform optimally.
- **Cell Culture Basics by the Best.**
  We offer chemicals, buffers, solvents, and antibiotics with the same high level of quality that we put into the rest of our portfolio.
- **Optimized Kits and Media Supplements.**
  With high quality growth factors and cytokines at our disposal, we've designed kits and media for the growth and differentiation of immune cells, brain cells, and stem cells.



N21-MAX Media Supplement Enhances Synaptic Development. E18 rat hippocampal neurons were grown for 19 days *in vitro* in media supplemented with either N21-MAX Media Supplement (R&D Systems, Catalog # AR008) or the neural media supplement from the most widely-used competitor. Cells were incubated with the synaptic vesicle dye SynaptoRed™ C2 (Tocris, Catalog # 5118), for 1 minute prior to depolarization with KCl. Cells were imaged for SynaptoRed™ C2-positive synaptic puncta. (**A**) Quantification of SynaptoRed™ C2-positive puncta shows that neurons grown in the N21-MAX Media Supplement have an increased number of synaptic puncta compared to the competitor media. (**B**) Quantification of dye intensity shows that neurons grown in the N21-MAX Media Supplement have more robust synaptic activity than neurons cultured in the competitor media. (**C**) Representative images of SynaptoRed™ C2 staining in neurons cultured in the N21-MAX Media Supplement or competitor media.



N-2 MAX Media Supplement Provides Optimal Growth Conditions for Neural Progenitor Cell Expansion. Human neural progenitor cells were maintained in culture with N-2 MAX Media Supplement (Catalog # AR009). The cells were then stained for SOX1 expression using a Goat Anti-Human SOX1 Antigen Affinity-Purified Polyclonal Antibody (Catalog # AF3369) followed by a NL557-Conjugated Donkey Anti-Goat IgG Secondary Antibody (Catalog # NL001; red). The cells were also stained for Nestin expression using a Mouse Anti-Mouse/Rat Nestin Monoclonal Antibody (Catalog # MAB2736) followed by a NL493-Conjugated Donkey Anti-Mouse IgG Secondary Antibody (Catalog # NL009; green). The cells were counterstained with DAPI (blue). All cited reagents are from R&D Systems.

Learn more | rndsystems.com/neuralculture

R&D Systems® Cell Culture Media and Supplements

Product	Catalog #	Description
N-2 Plus Media Supplement	AR003	Optimized for neural progenitor cells and their differentiated derivatives; contains bovine Insulin
N-2 MAX Media Supplement (100x)	AR009	Optimized for neural progenitor cells and their differentiated derivatives; contains recombinant human Insulin
N21-MAX Media Supplement (50x)	AR008	Superior growth supplement for the long-term culture of neurons; optimized formulation of the original B27 ingredients
N21-MAX Insulin Free Media Supplement (50x)	AR010	Growth supplement for insulin-sensitive neuronal cell cultures; optimized and insulin-free formulation of the original B27 ingredients
N21-MAX Vitamin A Free Media Supplement (50x)	AR012	For neural stem cell expansion; limits retinoic acid production to reduce unwanted differentiation
Holo-Transferrin	2914-HT	Facilitates iron transport and improves cell proliferation in culture

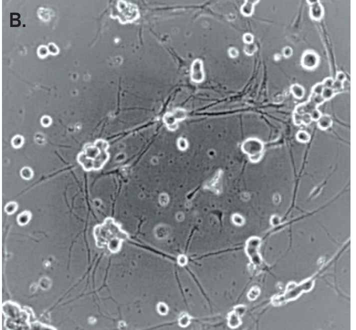
R&D Systems® Cell Culture Kits

Product	Catalog #	Description
NeuroXVivo™ Rat Cortical Neuron Culture Kit	CDK011	Optimized media and reagents for the maintenance and maturation of embryonic or postnatal rat cortical neurons in culture

Tocris® Antibiotics

Product	Catalog #
Ampicillin sodium salt	5503
Bafilomycin A1	1334
Blasticidin S	5502
Carbenicillin disodium salt	5507
Clindamycin	4822
Cycloheximide	0970
G418	4131
Ionomycin	1704
Kanamycin	5505
Leptomycin B	1987
Oligomycin	4110
Puromycin	4089
Tunicamycin	3516

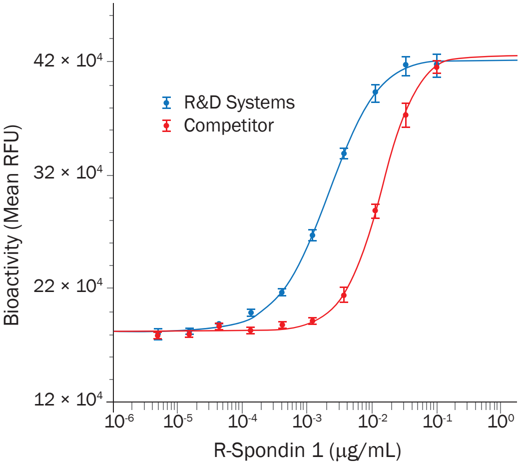
A.



F-Spondin-Induces Neurite Outgrowth. Embryonic chick dorsal root ganglion neurons (E13) were cultured in the absence (**A**) and presence (**B**) of Recombinant Human F-Spondin (R&D Systems, Catalog # 3135-SP) immobilized on a nitrocellulose-coated microplate. The presence of F-Spondin significantly enhanced neurite outgrowth.

Tocris® Buffers and Solutions

Product	Catalog #
ACSF	3525
L-Glutamine (Ala-Gln)	5823
BIS-TRIS	5216
Dulbecco's PBS	3156
HEPES	3173
HEPES Sodium salt	3174
PBS, 100 Tablets	5564
Water, sterile filtered	3179



R&D Systems® Human R-Spondin 1 Exhibits Greater Activity Compared to Competitor's. HEK293T cells were incubated with increasing concentrations of R&D Systems Recombinant Human R-Spondin 1 (Catalog # 4645-RS) and a competitor's R-Spondin 1 in the presence of 5 ng/mL Recombinant Mouse Wnt-3a (R&D Systems, Catalog # 1324-WN). R-Spondin 1 induced activation of β-Catenin in a dose dependent manner as measured using the Topflash assay. The activity of R&D Systems Recombinant Human R-Spondin 1 (blue) was approximately 7-fold greater than the competitor's R-Spondin 1 (red).

Cell Culture Products, continued

R&D Systems® Extracellular Matrix Molecules

Molecule	Species	Catalog #	Source
Agrin	Human	6624-AG	CHO
Agrin (N-Terminal)	Human	8909-AG	CHO
Agrin	Rat	550-AG	*Sf* 21
Decorin	Human	143-DE	*Sf* 21
Decorin	Mouse	1060-DE	NS0
Fibronectin	Human	1918-FN	Plasma
Fibronectin	Human	4305-FN	NS0
Fibronectin, ACFP	Human	ACFP4305	*Sf* 9
Fibronectin, GMP	Human	4305-GMP	*Sf* 9
Fibronectin, Fragment 2	Human	3225-FN	NS0
Fibronectin, Fragment 3	Human	3938-FN	NS0
Fibronectin, Fragment 4	Human	3624-FN	NS0
Fibronectin	Bovine	1030-FN	Plasma
Nidogen-1/Entactin	Human	2570-ND	NS0
F-Spondin/SPON1	Human	3135-SP	NS0
F-Spondin/SPON1	Mouse	7950-SP	NS0
R-Spondin 1	Human	4645-RS	CHO
R-Spondin 1, Biotinylated	Human	BT4645	CHO

Molecule	Species	Catalog #	Source
R-Spondin 1	Mouse	3474-RS	*E. coli*
R-Spondin 1	Mouse	7150-RS	CHO
R-Spondin 2	Human	3266-RS	NS0
R-Spondin 2	Mouse	6946-RS	CHO
R-Spondin 3	Human	3500-RS	CHO
R-Spondin 3	Mouse	4120-RS	CHO
R-Spondin 4	Human	4575-RS	CHO
R-Spondin 4	Mouse	4106-RS	CHO
Tenascin C	Human	3358-TC	NS0
Tenascin R	Human	3865-TR	NS0
Tenascin XB2	Human	6999-TN	CHO
Testican 1/SPOCK1	Human	2327-PI	NS0
Testican 2/SPOCK2	Human	2328-PI	NS0
Testican 3/SPOCK3	Mouse	2346-PI	NS0
Vitronectin	Human	2349-VN	Plasma
Vitronectin	Human	2308-VN	NS0
Vitronectin	Bovine	2348-VN	Plasma

R&D Systems® Additional Cell Adhesion Molecules

Product	Catalog #	Description
Cultrex® Poly-D-Lysine	3439-100-01	A highly positively charged synthetic amino acid chain; used as a coating agent to promote cell adhesion in culture
Cultrex Poly-L-Lysine	3438-100-01	A highly positively charged amino acid chain; used as a coating agent to promote cell adhesion in culture

R&D Systems® Basement Membrane Extracts (BME)

Product	Catalog #	Description
Cultrex® Bovine Collagen I	3442-050-01	Fetal bovine extensor tendon-derived protein that facilitates cell adherence to culture dishes and promotes cell spreading *in vitro*; provided at a concentration of 5 mg/mL
Cultrex Rat Collagen I	3440-100-01	Rat tail tendon-derived protein that facilitates cell adherence to culture dishes and promotes cell spreading *in vitro*; provided at a concentration of 5 mg/mL
Cultrex Rat Collagen I (Lower Viscosity)	3443-003-01	Rat tail tendon-derived protein that facilitates cell adherence to culture dishes and promotes cell spreading *in vitro*; provided at a concentration of 3 mg/mL
Cultrex 3D Culture Matrix Rat Collagen I	3447-020-01	Rat tail tendon-derived protein for use in 3D cultures; facilitates cell attachment and spreading *in vitro*; provided at a concentration of 5 mg/mL
Cultrex Mouse Collagen IV	3410-010-01	Murine EHS tumor-derived protein that facilitates cell adherence to culture dishes and promotes cell spreading *in vitro*; provided at a concentration of 0.5 mg/mL
Cultrex Mouse Laminin I	3400-010-01	Murine EHS tumor-derived protein that facilitates cell adherence to culture dishes; provided at a concentration of 1 mg/mL
Cultrex 3D Culture Matrix Laminin I	3446-005-01	Murine EHS tumor-derived protein for use in 3D cultures; facilitates cell attachment and spreading *in vitro*; provided at a concentration of 6 mg/mL
Cultrex PathClear® Basement Membrane Extract	3432-005-01	BME consisting of Laminin I, Collagen IV, Entactin, and Heparan Sulfate Proteoglycan purified from murine EHS tumor; confirmed negative by PCR for 31 infectious organisms and viruses; available in multiple volume sizes, with and without phenol red
Cultrex PathClear Reduced Growth Factor BME	3433-005-01	BME subjected to a special process to reduce growth factor content, ideal for studies focused on cell growth; confirmed negative by PCR for 31 infectious organisms and viruses; available in multiple volume sizes, with and without phenol red
Cultrex PathClear BME, Type 2	3532-005-02	BME that is formulated to exhibit higher tensile strength; confirmed negative by PCR for 31 infectious organisms and viruses; available in multiple volume sizes, with and without phenol red
Cultrex PathClear Reduced Growth Factor BME, Type 2	3533-005-02	BME with reduced growth factor content and higher tensile strength; confirmed negative by PCR for 31 infectious organisms and viruses; available in multiple volume sizes, with and without phenol red
Cultrex PathClear 3D Culture Matrix Reduced Growth Factor BME	3445-001-01	BME with reduced growth factor content, produced and qualified specifically for use in 3D culture studies; confirmed negative by PCR for 31 infectious organisms and viruses; available in multiple volume sizes
StemXVivo® Culture Matrix (100x)	CCM013	A defined matrix for the growth and differentiation of stem cells and progenitor cells

Abbreviation Key: ACFP (Animal Component-Free Process),
Source Key: CHO (Chinese hamster ovary cell line), NS0 (Mouse myeloma cell line), *Sf* (*Spodoptera frugiperda*)

Tocris® Chemicals and Laboratory Reagents

Product	Catalog #
Boric acid	3177
Bovine Serum Albumin	5217
Calcium chloride dihydrate	3148
CHAPS	3172
Citric acid	3160
D-(+)-Glucose	5504
DMSO, sterile filtered	3176
Glycerol	5220
Ponceau S Staining Solution	5225
Potassium Chloride	3147
Potassium phosphate monobasic	3150
Sodium acetate	3159
Sodium bicarbonate	3152
Sodium chloride	3146
Sodium citrate	3161
Sodium phosphate dibasic	3153
Sodium phosphate monobasic	5226
TRIS base	3163
TRIS hydrochloride	3164
Urea	5228

Tocris® Substrates and Chelators

Product	Catalog #
BAPTA	2786
BAPTA AM	2787
DAPI	5748
D-Luciferin sodium salt	5427
EDTA	2811
EGTA	2807
Hoechst 33342	5117
IPTG	5509
L 012 sodium salt	5085
MTT	5224
X-Gal	5510

R&D Systems® Culture Microplates

Product	Catalog #	Description
Clear Polystyrene Microplate	DY990	Uncoated, high-binding, flat-bottom microplate; 12 × 8 well strips with frame, 360 µL well volume; 25 plates/pack
Human Fibronectin-Coated Microplate	CWP001	Clear, polystyrene 96-well microplate, coated with 1 µg of human Fibronectin per well and/or blocked with BSA Fraction V under aseptic conditions; 5 plates/pack
Human Vitronectin-Coated Microplate	CWP003	Clear, polystyrene 96-well microplate, coated with 0.5 µg of human Vitronectin per well and/or blocked with BSA Fraction V under aseptic conditions; 5 plates/pack

R&D Systems® Mycoplasma Detection

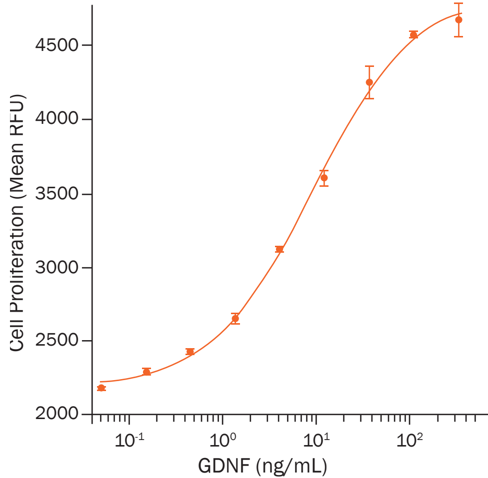
Product	Catalog #	Description
MycoProbe™ Mycoplasma Detection Kit	CUL001B	A complete kit to detect common antibiotic-resistant cell culture contaminants

R&D Systems® Proteins

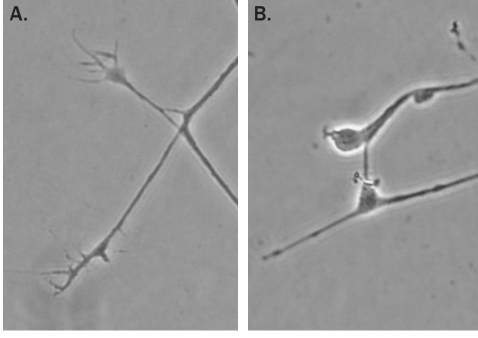
The Most Reputable Manufacturer

of over 4800 biologically active recombinant and natural proteins spanning 22 species.

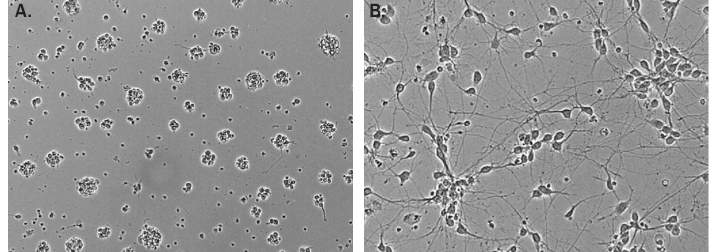
- **Guaranteed Performance, Always.**
  We back up our performance specifications with relevant bioassay data and every lot is tested before release to ensure consistent performance.
- **Custom Development and GMP Manufacturing.**
  We can make proteins to meet your specifications, including manufacturing using GMP guidelines for cell therapy.
- **Manufactured by Scientists, for Scientists.**
  Our team of over 100 protein scientists brings the most experience and know-how in the industry to the development and production of our proteins.



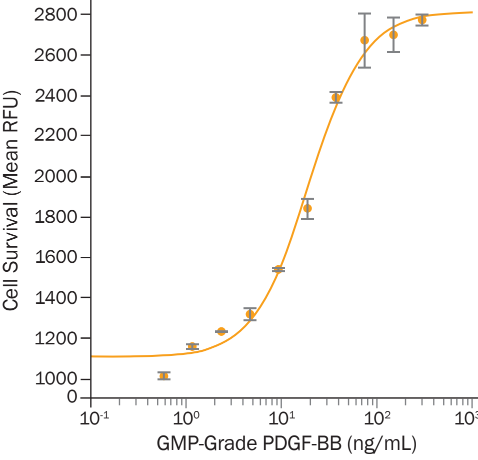
GDNF Induces Proliferation of Human Neuroblastoma Cells. The SH-SY5Y human neuroblastoma cell line was treated with increasing concentrations of Recombinant Human GDNF (Catalog # 212-GD). Cell proliferation was assessed using Resazurin (Catalog # AR002). All cited reagents are from R&D Systems.



Semaphorin 3A-Induced Growth Cone Collapse. A fully extended chick dorsal root ganglion growth cone, grown in the presence of Recombinant Human β-NGF (Catalog # 256-GF), was untreated (**A**) or treated (**B**) with Recombinant Human Semaphorin 3A (Catalog # 1250-S3). Treatment with Semaphorin 3A induced growth cone collapse. All cited reagents are from R&D Systems.



Slit3 Stimulates Neurite Outgrowth. Embryonic rat cortical neurons (E16-E18) were cultured in the absence (**A**) or presence (**B**) of Recombinant Human Slit3 (R&D Systems, Catalog # 9067-SL). The presence of the Slit3 protein significantly enhanced neurite outgrowth.



Bioactivity of GMP-Grade Recombinant Human PDGF-BB. GMP-Grade Recombinant Human PDGF-BB (Catalog # 220-GMP) enhances survival of rat embryonic cortical neurons (E16–E18). Cell survival was assessed using Resazurin (Catalog # AR002). All cited reagents are from R&D Systems.

Learn more | rndsystems.com/neuralproteins

R&D Systems® Neurotrophic and Neuronal Differentiation Factors

Activins

Molecule	Species	Catalog #	Source
Activin A	Human, Mouse, Rat	338-AC	CHO
Activin A, ACFP	Human, Mouse, Rat	ACFP338	*Sf* 9
Activin A, GMP	Human, Mouse, Rat	338-GMP	CHO
Activin A, ProDots®	Human, Mouse, Rat	PRD338	CHO
Latent Activin A	Human	9129-LA	CHO
Activin AB	Human	1066-AB	CHO
Activin AC	Human	4879-AC	CHO

Molecule	Species	Catalog #	Source
Activin B	Human	659-AB	CHO
Activin B	Mouse	8260-AB	HEK293
Activin C	Human	1629-AC	HEK293
Activin C	Mouse	489-AC	CHO
Inhibin α	Human	8506-AB	CHO
Inhibin α	Mouse	8346-IN	CHO

BMPs (Bone Morphogenetic Proteins)

Molecule	Species	Catalog #	Source
BMP-1/PCP	Human	1927-ZN	NS0
BMP-2	Human, Mouse, Rat	355-BEC	*E. coli*
BMP-2	Human, Mouse, Rat	355-BM	CHO
BMP-2, GMP	Human	355-GMP	CHO
BMP-2/BMP-6 Heterodimer	Human	7145-BP	*E. coli*
BMP-2/BMP-7 Heterodimer	Human	3229-BM	*E. coli*
BMP-3	Human	113-BP	*E. coli*
BMP-3b/GDF-10	Human	1543-BP	*E. coli*
BMP-4	Human	314-BP	NS0
BMP-4, GMP	Human	314-GMP	NS0
BMP-4, ProDots®	Human	PRD314	NS0
BMP-4	Mouse	5020-BP	CHO
BMP-4/BMP-7 Heterodimer	Human	3727-BP	*E. coli*
BMP-5	Human	615-BMC	CHO
BMP-5	Mouse	6176-BM	CHO
BMP-6	Human	507-BP	NS0

Molecule	Species	Catalog #	Source
BMP-6	Human	BT507	NS0
BMP-6	Mouse	6325-BM	CHO
BMP-7	Human	354-BP	CHO
BMP-7	Human	BT354	CHO
BMP-7, GMP	Human	354-GMP	CHO
BMP-7	Mouse	5666-BP	CHO
BMP-8a	Human	1073-BP	*E. coli*
BMP-8a	Human	1073-BPC	CHO
BMP-8a	Mouse	7540-BP	CHO
BMP-9	Human	3209-BP	CHO
BMP-9	Mouse	5566-BP	CHO
BMP-10	Human	2926-BP	CHO
BMP-10	Mouse	6038-BP	CHO
BMP-10 Propeptide	Human	3956-BP	NS0
BMP-15/GDF-9B	Human	5096-BM	CHO

EGF Ligands

Molecule	Species	Catalog #	Source
Amphiregulin	Human	262-AR	*E. coli*
Amphiregulin	Mouse	989-AR	*E. coli*
Betacellulin/BTC	Human	261-CE	*E. coli*
Betacellulin/BTC	Mouse	1025-CE	*E. coli*
EGF	Human	236-EG	*E. coli*
EGF, GMP	Human	236-GMP	*E. coli*
EGF, ProDots®	Human	PRD236	*E. coli*
EGF	Mouse	2028-EG	*E. coli*
EGF	Rat	3214-EG	*E. coli*
Pro-EGF (aa 21–1023)	Human	4289-EG	NS0
Pro-EGF (aa 29–1029)	Mouse	4095-EG	NS0
EGF-L6	Human	8638-EG	HEK293
EGF-L6	Mouse	4329-EG	NS0
Epigen	Human	6629-EP	*E. coli*

Molecule	Species	Catalog #	Source
Epigen	Mouse	1127-EP	*E. coli*
Epiregulin	Human	1195-EP	*E. coli*
Epiregulin	Mouse	1068-EP	*E. coli*
HB-EGF	Human	259-HE	*Sf* 21
LRIG1	Human	8504-LR	HEK293
LRIG1	Mouse	3688-LR	NS0
LRIG3	Human	3495-LR	NS0
Neuregulin-1/NRG1	Human	5898-NR	NS0
NRG1 (Isoform SMDF)	Human	378-SM	*Sf* 21
NRG1-α/HRG1-α (EGF Domain)	Human	296-HR	*E. coli*
NRG1-β1/HRG1-β1 (ECD)	Human	377-HB	*E. coli*
NRG1-β1/HRG1-β1 (EGF Domain)	Human	396-HB	*E. coli*
NRG1-β1/HRG1-β1, GMP	Human	396-GMP	*E. coli*
TGF-α	Human	239-A	*E. coli*

Abbreviation Key: ACFP (Animal Component-Free Process), ECD (Extracellular Domain), TC (Tissue Culture)
Source Key: CHO (Chinese hamster ovary cell line), HEK293 (Human embryonic kidney cell line), NS0 (Mouse myeloma cell line), *Sf* (*Spodoptera frugiperda*)

Neurotrophic and Neuronal Differentiation Factors, continued

FGF Ligands

Molecule	Species	Catalog #	Source
FGF acidic	Mouse	4686-FA	*E. coli*
FGF acidic	Bovine	132-FA	Bovine Brain
FGF acidic, Animal-Free	Human	AFL232	*E. coli*
FGF acidic (aa 2–155)	Human	231-BC	*E. coli*
FGF acidic (aa 16–155)	Human	232-FA	*E. coli*
FGF basic	Mouse	3139-FB	*E. coli*
FGF basic	Rat	3339-FB	*E. coli*
FGF basic	Bovine	133-FB	Bovine Brain
FGF basic	Bovine	2099-FB	*E. coli*
FGF basic, Animal-Free	Bovine	AFL2099	*E. coli*
FGF basic (145 aa)	Human	3718-FB	*E. coli*
FGF basic (145 aa, TC Grade)	Human	4114-TC	*E. coli*
FGF basic (145 aa), GMP	Human	3718-GMP	*E. coli*
FGF basic (146 aa)	Human	233-FB	*E. coli*
FGF basic (146 aa), GMP	Human	233-GMP	*E. coli*
FGF basic (146 aa), ProDots®	Human	PRD233	*E. coli*
FGF basic (157 aa)	Human	234-FSE	*E. coli*
FGF-3	Human	1206-F3	*E. coli*
FGF-3, ProDots	Human	PRD1206	*E. coli*
FGF-4	Human	235-F4	*E. coli*
FGF-4	Mouse	5846-F4	*E. coli*
FGF-4 (aa 67–202)	Mouse	7486-F4	*E. coli*
FGF-4 (aa 71–206)	Human	7460-F4	*E. coli*
FGF-5	Human	237-F5	*E. coli*
FGF-6	Human	238-F6	*E. coli*
FGF-6	Mouse	5750-F6	*E. coli*
FGF-6 (aa 67–208)	Human	6829-F6	*E. coli*

Molecule	Species	Catalog #	Source
KGF/FGF-7	Human	251-KG	*E. coli*
KGF/FGF-7, GMP	Human	251-GMP	*E. coli*
KGF/FGF-7, ProDots	Human	PRD251	*E. coli*
KGF/FGF-7	Mouse	5028-KG	*E. coli*
FGF-8a	Human	4745-F8	*E. coli*
FGF-8b	Human, Mouse	423-F8	*E. coli*
FGF-8c	Mouse	424-FC	*E. coli*
FGF-8e	Human	4746-F8	*E. coli*
FGF-8f	Human	5027-FF	*E. coli*
FGF-9	Human	273-F9	*Sf* 21
FGF-9	Mouse	7399-F9	*E. coli*
FGF-10	Human	345-FG	*E. coli*
FGF-10, ProDots	Human	PRD345	*E. coli*
FGF-10	Mouse	6224-FG	*E. coli*
FGF-10	Rat	7804-FG	*E. coli*
FGF-12	Human	2246-FG	*E. coli*
FGF-16	Human	1212-FG	*E. coli*
FGF-17	Human	319-FG	*E. coli*
FGF-17	Mouse	7400-FG	*E. coli*
FGF-18	Human	8988-F18	*E. coli*
FGF-19	Human	969-FG	*E. coli*
FGF-20	Human	2547-FG	*E. coli*
FGF-21	Human	2539-FG	*E. coli*
FGF-21	Mouse	8409-FG	NS0
FGF-22	Human	3867-FG	*E. coli*
FGF-23	Human	2604-FG	NS0
FGF-23	Mouse	2629-FG	NS0

GDNF Family Ligands

Molecule	Species	Catalog #	Source
Artemin	Human	2589-AR	*E. coli*
Artemin	Mouse	1085-AR	*E. coli*
GDNF	Human	212-GD	NS0
GDNF, GMP	Human	212-GMP	NS0
GDNF	Rat	512-GF	*Sf* 21

Molecule	Species	Catalog #	Source
Neurturin	Human	1297-NE	*E. coli*
Neurturin (Histidine-tagged)	Human	387-NE	*E. coli*
Neurturin	Mouse	477-MN	*E. coli*
Persephin	Human	2388-PS	*E. coli*
Persephin	Mouse	2479-PS	*E. coli*

Hedgehogs

Molecule	Species	Catalog #	Source
Desert Hedgehog (C23II, N-Terminus)	Mouse	733-DH	*E. coli*
Desert Hedgehog (N-Terminus)	Human	4777-DH	*E. coli*
Indian Hedgehog (C28II, N-Terminus)	Human, Mouse	1705-HH	*E. coli*
Sonic Hedgehog (High Activity)	Human	8908-SH	HEK293
Sonic Hedgehog (C24II, N-Terminus)	Human	1845-SH	*E. coli*

Molecule	Species	Catalog #	Source
Sonic Hedgehog (C24II, N-Terminus), GMP	Human	1845-GMP	*E. coli*
Sonic Hedgehog (C25II, N-Terminus)	Mouse	464-SH	*E. coli*
Sonic Hedgehog (N-Terminus)	Human	1314-SH	*E. coli*
Sonic Hedgehog (N-Terminus), GMP	Human	1314-GMP	*E. coli*
Sonic Hedgehog (N-Terminus)	Mouse	461-SH	*E. coli*

IGF Ligands

Molecule	Species	Catalog #	Source
IGF-I	Human	291-G1	*E. coli*
IGF-I, Animal-Free	Human	AFL291	*E. coli*
IGF-I, GMP	Human	291-GMP	*E. coli*
IGF-I, ProDots®	Human	PRD291	*E. coli*
IGF-I	Mouse	791-MG	*E. coli*
IGF-I	Rat	4326-RG	*E. coli*

Molecule	Species	Catalog #	Source
LR3 IGF-I	Human	8335-G1	*E. coli*
LR3 IGF-I, GMP	Human	8335-GMP	*E. coli*
LR3 IGF-I, ProDots	Human	PRD8335	*E. coli*
IGF-II	Human	292-G2	*E. coli*
IGF-II	Mouse	792-MG	*E. coli*

Neurotrophin/Trk Family Ligands

Molecule	Species	Catalog #	Source
BDNF	Human, Mouse, Rat, Canine, Equine	248-BD	*Sf* 21
BDNF, GMP	Human, Mouse, Rat, Canine, Equine	248-GMP	*Sf* 21
β-NGF	Human	256-GF	NS0
β-NGF	Mouse	1156-NG	NS0
β-NGF	Rat	556-NG	*Sf* 21

Molecule	Species	Catalog #	Source
β-NGF	Rat	7815-NG	CHO
NT-3	Human	267-N3	*Sf* 21
NT-3, GMP	Human	267-GMP	*Sf* 21
NT-4	Human	268-N4	*Sf* 21
NT-4, GMP	Human	268-GMP	*Sf* 21
NT-4	Mouse	3236-N4	NS0

PDGF Family Ligands

Molecule	Species	Catalog #	Source
PDGF	Human	120-HD	Human Platelets
PDGF-AA	Human	221-AA	*E. coli*
PDGF-AA	Rat	1055-AA	*E. coli*
PDGF-AB	Human	222-AB	*E. coli*
PDGF-AB	Rat	1115-AB	*E. coli*
PDGF-BB	Human	220-BB	*E. coli*
PDGF-BB, Biotinylated	Human	BT220	*E. coli*

Molecule	Species	Catalog #	Source
PDGF-BB, GMP	Human	220-GMP	*E. coli*
PDGF-BB, ProDots®	Human	PRD220	*E. coli*
PDGF-BB	Rat	520-BB	*E. coli*
PDGF-CC	Human	1687-CC	*E. coli*
PDGF-CC	Mouse	1447-PC	*E. coli*
PDGF-DD	Human	1159-SB	NS0

TGF-β Family Ligands

Molecule	Species	Catalog #	Source
LAP (TGF-β1)	Human	246-LP	*Sf* 21
Latent TGF-β1	Human	299-LT	CHO
TGF-β1	Human	100-B	Human Platelets
TGF-β1	Human	240-B	CHO
TGF-β1	Human	7754-BH	HEK293
TGF-β1, ACFP	Human	ACFP240	*Sf* 9
TGF-β1, GMP	Human	240-GMP	CHO
TGF-β1, ProDots®	Human	PRD240	CHO

Molecule	Species	Catalog #	Source
TGF-β1	Mouse	7666-MB	CHO
TGF-β1.2	Human	304-B3	*Sf* 21
TGF-β2	Human	302-B2	NS0
TGF-β2, ACFP	Human	ACFP302	*Sf* 9
TGF-β2	Mouse	7346-B2	CHO
TGF-β3	Human	243-B3	*Sf* 21
TGF-β3	Human	8420-B3	CHO
TGF-β3, GMP	Human	243-GMP	*Sf* 9

Abbreviation Key: ACFP (Animal Component-Free Process), ECD (Extracellular Domain), TC (Tissue Culture)
Source Key: CHO (Chinese hamster ovary cell line), HEK293 (Human embryonic kidney cell line), NS0 (Mouse myeloma cell line), *Sf* (*Spodoptera frugiperda*)

Neurotrophic and Neuronal Differentiation Factors, continued

VEGF Family Ligands

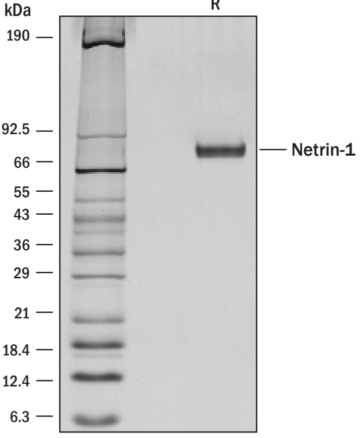
Molecule	Species	Catalog #	Source
PlGF	Human	264-PGB	*E. coli*
PlGF-2	Mouse	465-PL	*Sf* 21
PlGF-2	Human	6837-PL	CHO
PlGF-3	Human	7758-PL	*Sf* 21
PlGF-4	Human	8987-PL	HEK293
VEGF/PlGF Heterodimer	Human	297-VP	*E. coli*
VEGF, ACFP	Human	ACFP293	*Sf* 9
VEGF (aa 207–318)	Human	298-VS	*E. coli*
VEGF 111	Human	5336-VE	*E. coli*
VEGF 120	Mouse	494-VE	*E. coli*
VEGF 121 (aa 207–327)	Human	4644-VS	*E. coli*
VEGF 145 (aa 27–171)	Human	7626-VE	*E. coli*
VEGF 162	Human	2347-VE	NS0
VEGF 164	Mouse	493-MV	*Sf* 21
VEGF 164	Rat	564-RV	NS0

Molecule	Species	Catalog #	Source
VEGF 165	Human	293-VE	*Sf* 21
VEGF 165	Human	BT293	*Sf* 21
VEGF 165 (Extended Isoform)	Human	9018-VE	CHO
VEGF 165, GMP	Human	293-GMP	*Sf* 9
VEGF 165, ProDots®	Human	PRD293	*Sf* 21
VEGF 165b	Human	3045-VE	*Sf* 21
VEGF 188	Mouse	7916-MV	HEK293
VEGF 189 (aa 27–215)	Human	8147-VE	CHO
VEGF-B 167	Human	751-VE	*E. coli*
VEGF-B 167	Mouse	2595-VE	*E. coli*
VEGF-B 186	Mouse	767-VE	*Sf* 21
VEGF-C	Human	2179-VC	NS0
VEGF-C (Cys156Ser)	Human	752-VC	NS0
VEGF-D	Human	622-VD	*Sf* 21
VEGF-D	Mouse	469-VD	*Sf* 21

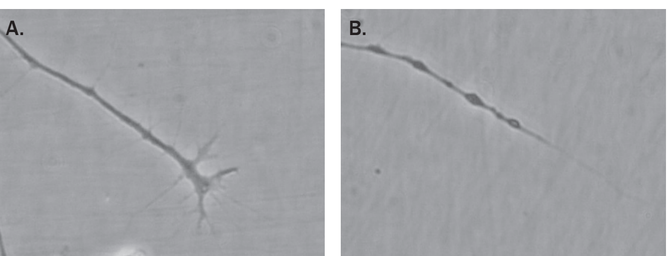
Wnt Ligands

Molecule	Species	Catalog #	Source
Wnt-2b	Mouse	3900-WN	CHO
Wnt-3a	Human	5036-WN	CHO
Wnt-3a, GMP	Human	5036-GMP	CHO
Wnt-3a	Mouse	1324-WN	CHO
Wnt-3a (High Purity)	Mouse	1324-WNP	CHO
Wnt-4	Human	6076-WN	CHO
Wnt-4	Mouse	475-WN	CHO
Wnt-5a	Human, Mouse	645-WN	CHO
Wnt-5b	Human	7347-WN	CHO

Molecule	Species	Catalog #	Source
Wnt-5b	Mouse	3006-WN	CHO
Wnt-7a	Human	3008-WN	CHO
Wnt-8a	Mouse	8419-WN	CHO
Wnt-9a	Mouse	8148-WN	CHO
Wnt-9b	Mouse	3669-WN	CHO
Wnt-10b	Human	7196-WN	CHO
Wnt-10b	Mouse	2110-WN	CHO
Wnt-11	Human	6179-WN	CHO
Wnt-16b	Human	7790-WN	CHO



R&D Systems® Netrin-1 Protein is Highly Pure. To highlight purity, Recombinant Mouse Netrin-1 (R&D Systems, Catalog # 1109-N1) was visualized on a silver stained 4–20% SDS polyacrylamide gel under reducing (**R**) conditions. A single band is observed at approximately 83 kDa (as indicated).



Semaphorin 6B-Induced Growth Cone Collapse. A fully extended chick dorsal root ganglion growth cone, grown in the presence of Recombinant Human β-NGF (Catalog # 256-GF), was untreated (**A**) or treated (**B**) with Recombinant Human Semaphorin 6B (Catalog # 2094-S6). Treatment with Semaphorin 6B induced growth cone collapse. All cited reagents are from R&D Systems.

R&D Systems® Axon Guidance Cues

Netrins

Molecule	Species	Catalog #	Source
Netrin-1	Human	6419-N1	NS0
Netrin-1	Mouse	1109-N1	NS0
Netrin-1	Chicken	128-N1	NS0
Netrin-2	Chicken	127-N2	NS0
Netrin-4	Human	1254-N4	NS0

Molecule	Species	Catalog #	Source
Netrin-4	Mouse	1132-N4	NS0
Netrin-G1a	Mouse	1166-NG	*Sf* 21
Netrin-G2a	Mouse	2744-NG	NS0
NGL-1/LRRC4C	Human	4899-NR	NS0

Semaphorins

Molecule	Species	Catalog #	Source
Semaphorin 3A	Human	1250-S3	NS0
Semaphorin 3A	Mouse	5926-S3	CHO
Semaphorin 3B	Mouse	5440-S3	NS0
Semaphorin 3C	Human	5570-S3	NS0
Semaphorin 3C (Truncated)	Mouse	1728-S3	*Sf* 21
Semaphorin 3E	Human	3239-S3B	NS0
Semaphorin 3E	Mouse	3238-S3	*Sf* 21
Semaphorin 3F (Truncated)	Mouse	3237-S3	NS0
Semaphorin 4A	Human	4694-S4	NS0
Semaphorin 4C	Human	6125-S4	NS0
Semaphorin 4C	Mouse	8394-S4	NS0
Semaphorin 4D/CD100	Human	7470-S4	CHO
Semaphorin 4D/CD100	Mouse	5235-S4	NS0
Semaphorin 4F	Mouse	7200-S4	NS0
Semaphorin 4G	Human	5840-S4	NS0

Molecule	Species	Catalog #	Source
Semaphorin 4G	Mouse	6504-S4	NS0
Semaphorin 5A	Human	5896-S5	NS0
Semaphorin 5A	Mouse	6584-S5	CHO
Semaphorin 5B	Human	6680-S5	CHO
Semaphorin 5B	Mouse	6766-S5	CHO
Semaphorin 6A	Human	1146-S6	NS0
Semaphorin 6A	Mouse	9017-S6	*Sf* 21
Semaphorin 6B	Human	2094-S6	NS0
Semaphorin 6B	Mouse	2264-S6	HEK293
Semaphorin 6C	Human	2219-S6	NS0
Semaphorin 6C	Mouse	2108-S6	NS0
Semaphorin 6D	Human	2095-S6	NS0
Semaphorin 6D	Mouse	7067-S6	NS0
Semaphorin 7A	Human	2068-S7	NS0
Semaphorin 7A	Mouse	1835-S3	NS0

Slit Ligands

Molecule	Species	Catalog #	Source
Slit1	Human	6514-SL	CHO
Slit1	Mouse	5199-SL	CHO
Slit2	Human	8616-SL	HEK293

Molecule	Species	Catalog #	Source
Slit2	Mouse	5444-SL	CHO
Slit3 (aa 1120–1523)	Human	9067-SL	HEK293

SLITRK Family

Molecule	Species	Catalog #	Source
SLITRK1	Human	3009-SK	NS0
SLITRK2	Human	8947-SK	NS0
SLITRK3	Human	8957-SK	NS0

Molecule	Species	Catalog #	Source
SLITRK4	Human	8945-SK	NS0
SLITRK5	Human	2587-SK	NS0

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Abbreviation Key: ACFP (Animal Component-Free Process), ECD (Extracellular Domain), TC (Tissue Culture)
Source Key: CHO (Chinese hamster ovary cell line), HEK293 (Human embryonic kidney cell line), NS0 (Mouse myeloma cell line), *Sf* (*Spodoptera frugiperda*)

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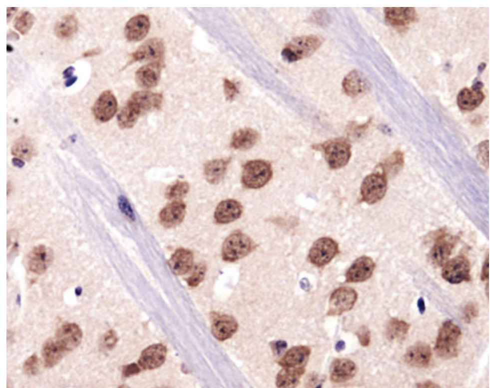
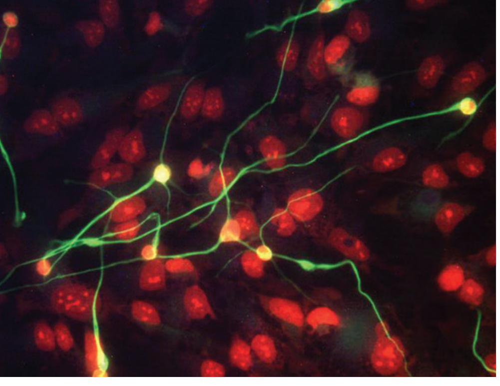
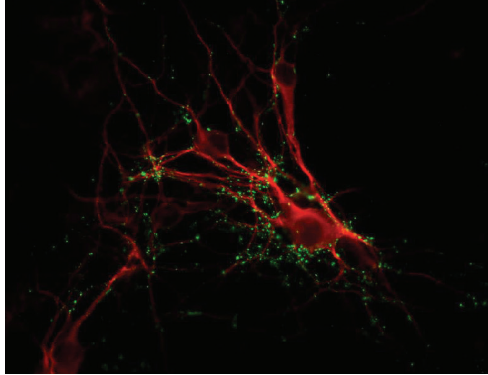


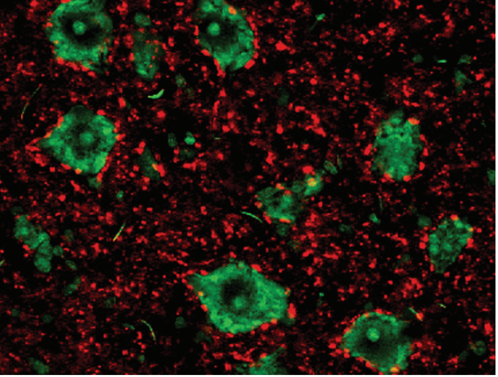
NeuN in Mouse Brain. NeuN was detected in immersion-fixed paraffin-embedded sections of mouse brain (cerebellum) using Rabbit Anti-Human/Mouse/Rat NeuN Antigen Affinity-Purified Polyclonal Antibody (Novus Biologicals, Catalog # NBP1-77686). The cerebellar tissue was stained using HRP and DAB (brown), and counterstained with hematoxylin (blue).



βIII Tubulin in Differentiated Human Neural Progenitor Cells. βIII Tubulin was detected in immersion-fixed differentiated human neural progenitor cells using a Mouse Anti-Neuron-Specific βIII Tubulin (Clone TuJ-1) Monoclonal Antibody (R&D Systems, Catalog # MAB1195). The cells were stained (green) and counterstained (red).



Synapsin I in Rat Caudate Neurons. Synapsin I was detected in fixed rat caudate neurons using a Rabbit Anti-Human/Mouse/Rat/Bovine/Guinea Pig/Primate Synapsin I Polyclonal Antibody (Novus Biologicals, Catalog # NB300-104). The cells were stained (green) and then counterstained for MAP proteins (red).



Synaptotagmin-1 in Rat Spinal Cord. Synaptotagmin-1 was detected in perfusion-fixed frozen sections of rat spinal cord using a Mouse Anti-Rat Synaptotagmin-1 Monoclonal Antibody (R&D Systems, Catalog # MAB43641). The tissue was stained (red) and counterstained (green).

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Neuronal Markers

Marker	Catalog #	Brand	Species	Clonality	Applications	Conjugate
Calbindin D-28K	NBP2-50048	Novus Biologicals	Human, Mouse, Rat +	Mono	WB, ICC	No
Calbindin D-28K	AF3320	R&D Systems	Human	Poly	WB, IHC	No
Doublecortin (DCX)	NBP1-92684	Novus Biologicals	Human, Mouse, Rat +	Mono	WB, ICC	Yes
MAP2	NBP1-92711	Novus Biologicals	Human, Mouse, Rat +	Mono	WB, ICC	Yes
NeuN	NBP1-77686	Novus Biologicals	Human, Mouse, Rat	Poly	ICC, IHC	Yes
NeuroD1	AF2746	R&D Systems	Human, Mouse	Poly	WB, ICC	Yes
NF-H	NB300-135	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, ELISA	No
NF-L	NB300-131	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC	No
NF-M	NB300-133	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC	No
NSE	AF5169	R&D Systems	Human, Mouse	Poly	WB, IHC, SW	No
Tau	AF3494	R&D Systems	All Species	Poly	WB, IHC	No
β-III Tubulin	NB100-1612	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
β-III Tubulin (Clone TuJ-1), Neuron-Specific	MAB1195	R&D Systems	All Species	Mono	WB, ICC, SW	Yes
UCH-L1/PGP9.5	AF6007	R&D Systems	Human, Mouse, Rat	Poly	WB, SW	No

Synaptic Markers

Marker	Catalog #	Brand	Species	Clonality	Applications	Conjugate
Bassoon	NB120-13249	Novus Biologicals	Mouse, Rat	Mono	WB, ICC, IHC, IP	No
CaMKIIα	NB100-1983	Novus Biologicals	Human, Mouse, Rat +	Mono	WB, ICC, IHC, ELISA, IP, RI	Yes
DLG1	NBP1-48054	Novus Biologicals	Human, Mouse, Rat	Mono	WB, ICC, IHC, IP	No
Drebrin 1	MAB7739	R&D Systems	Human	Mono	WB, ICC, SW	No
EAAT1/GLAST-1/SLC1A3	NB100-1869	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, ELISA, FC,	Yes
GAP-43	NB300-143	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC	Yes
Gephyrin/GPHN	MAB7519	R&D Systems	Human, Mouse	Mono	WB, IHC	No
HOMER1	NBP1-44999	Novus Biologicals	Human, Mouse	Poly	WB, ICC	No
Neuroligin 1	AF4340	R&D Systems	Human, Rat	Poly	WB, IHC	Yes
NMDA Receptor 2B	NB300-106	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC, FC, IP	No
PPP1R9B/Spinophilin	AF6465	R&D Systems	Human, Rat	Poly	WB, ICC	No
PSD-93	NB300-546	Novus Biologicals	Rat	Poly	WB, ICC, IHC	No
PSD-95	NB300-556	Novus Biologicals	Human, Mouse, Rat +	Mono	WB, ICC, IHC, B/N, ChIP, FC, IP	Yes
SAP102	NBP1-87691	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
Shank1	NB300-167	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
Shank2	AF7035	R&D Systems	Human	Poly	IHC	No
Shank3	NBP1-47610	Novus Biologicals	Human, Mouse, Rat	Mono	WB, IHC, IP	No
SNAP25	AF5946	R&D Systems	Human, Mouse, Rat	Poly	WB, ICC	No
Synapsin I	NB300-104	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC, IP	No
Synaptophysin	NBP2-25170	Novus Biologicals	Human, Mouse +	Poly	WB, ICC, IHC	No
Synaptotagmin-1	MAB43641	R&D Systems	Rat	Mono	WB, IHC, IP	No
Synuclein-α	MAB13381	R&D Systems	Human, Mouse, Rat	Mono	WB, IHC	No
VAMP-1	AF4828	R&D Systems	Human, Mouse	Poly	WB, IHC	No
VAMP-2	MAB5136	R&D Systems	Human, Mouse	Mono	WB, IHC	No

Species Key: + Additional Species Available

Applications Key: B/N (Blocking/Neutralization), ChIP (Chromatin Immunoprecipitation), ELISA (ELISA Capture and/or Detection), EM (Electron Microscopy), FA (Functional Assay), FC (Flow Cytometry), GS (Gel Shift), ICC (Immunocytochemistry), IHC (Immunohistochemistry), IP (Immunoprecipitation), IV (*In Vitro*), PEP-ELISA (Peptide-ELISA), RI (Radioimmunoassay), SW (Simple Western™), and WB (Western blot)

Antibodies, continued

Markers for Glutamatergic Neurons

Marker	Catalog #	Brand	Species	Clonality	Applications	Conjugate
EAAT3	NBP1-84938	Novus Biologicals	Human	Poly	ICC, IHC	No
Glutaminase	NBP2-29940	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC, FC	No
Glutamine Synthetase	NB110-41404	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, IHC	No
GRIN1/NMDAR1	NB300-118	Novus Biologicals	Human, Mouse, Rat	Mono	WB, ICC, IHC, IP	No
GRIN2B/NMDAR2B	NB300-106	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC, FC, IP	No
VGLUT1/SLC17A7	NBP2-46627	Novus Biologicals	Human, Mouse, Rat	Mono	WB, IHC	No
VGLUT1/SLC17A7	MAB9054	R&D Systems	Human	Mono	IHC	No
VGLUT2/SLC17A6	NBP2-46641	Novus Biologicals	Human, Mouse, Rat	Mono	IHC	No
VGluT3/Slc17a8	NBP1-25973	Novus Biologicals	Mouse, Rat	Poly	WB, IHC	No

Markers for GABAergic Neurons

Marker	Catalog #	Brand	Species	Clonality	Applications	Conjugate
ABAT (GABA Transaminase)	NBP2-21598	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
DARPP-32	MAB4230	R&D Systems	Mouse, Rat	Mono	WB, IHC	No
GABA-B R1	AF7000	R&D Systems	Human, Mouse, Rat	Poly	WB, IHC	No
GABA-B R2 (N-Terminus)	AF1188	R&D Systems	Rat	Poly	WB, IHC	No
GAD1/GAD67	NBP2-46639	Novus Biologicals	Human, Mouse, Rat	Mono	WB, IHC	No
GAD1/GAD67	AF2086	R&D Systems	Human, Mouse, Rat	Poly	WB, IHC, SW	Yes
GAD2/GAD65	NBP1-33284	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC	No
GAD2/GAD65	AF2247	R&D Systems	Human	Poly	WB, IHC	No
GAT-1/SLC6A1	NBP1-89802	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
VIAAT/SLC32A1/VGAT	NBP2-20857	Novus Biologicals	Human, Mouse	Poly	WB, IHC	No

Markers for Dopaminergic Neurons

Marker	Catalog #	Brand	Species	Clonality	Applications	Conjugate
Dopamine β-Hydroxylase	NBP1-31386	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
HNF-3β/FoxA2	AF2400	R&D Systems	Human	Poly	WB, ICC, ChIP	Yes
LMX1b	NBP2-41194	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC, ELISA	No
Nurr1/NGFI-Bβ/NR4A2	AF2156	R&D Systems	Human, Mouse	Poly	WB, IHC	No
Parkin	NB300-429	Novus Biologicals	Human	Poly	WB, IP	No
Slc6a2/NET/Noradrenaline Transporter	NBP1-28665	Novus Biologicals	Mouse, Rat +	Mono	WB	No
Slc6a3/DAT1	NBP2-22164	Novus Biologicals	Mouse, Rat, Human (–ve)	Mono	WB, ICC, IHC, ELISA, IP	Yes
TorsinA	NBP2-20685	Novus Biologicals	Human	Poly	WB	No
Tyrosine Hydroxylase	NB300-109	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, SW	No
Tyrosine Hydroxylase	AF7566	R&D Systems	Human, Mouse, Rat	Poly	WB, ICC, SW	No

Markers for Glycinergic Neurons

Marker	Catalog #	Brand	Species	Clonality	Applications	Conjugate
GlyT2/SLC6A5	NBP1-59657	Novus Biologicals	Human	Poly	WB	No
VIAAT/SLC32A1/VGAT	NBP2-20857	Novus Biologicals	Human, Mouse	Poly	WB, IHC	No

Species Key: + Additional Species Available

Applications Key: B/N (Blocking/Neutralization), ChIP (Chromatin Immunoprecipitation), ELISA (ELISA Capture and/or Detection), EM (Electron Microscopy), FA (Functional Assay), FC (Flow Cytometry), GS (Gel Shift), ICC (Immunocytochemistry), IHC (Immunohistochemistry), IP (Immunoprecipitation), IV (*In Vitro*), PEP-ELISA (Peptide-ELISA), RI (Radioimmunoassay), SW (Simple Western™), and WB (Western blot)

Markers for Serotonergic Neurons

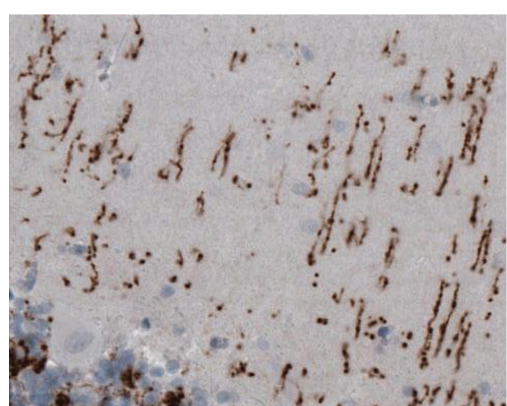
Marker	Catalog #	Brand	Species	Clonality	Applications	Conjugate
Dopa Decarboxylase/DCC	AF3564	R&D Systems	Human, Mouse, Rat	Poly	WB, ICC, IHC, IP	Yes
Monoamine Oxidase B	NBP1-87493	Novus Biologicals	Human, Mouse, Rat	Poly	WB, IHC	No
Slc6a4/5-HTTLPR/Serotonin Transporter	NBP1-78989	Novus Biologicals	Mouse, Rat	Mono	IHC, WB (–ve)	Yes
Tryptophan Hydroxylase 1/TPH-1	NB300-176	Novus Biologicals	Human, Rat, Rabbit (–ve)	Poly	WB, IHC	No
Tryptophan Hydroxylase 1/TPH-1	AF5276	R&D Systems	Human	Poly	WB, IHC	No
VMAT2	NB110-68123	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, IHC	No

Markers for Cholinergic Neurons

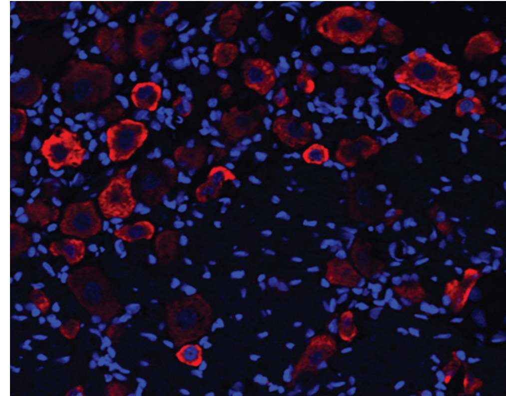
Marker	Catalog #	Brand	Species	Clonality	Applications	Conjugate
Acetylcholinesterase/ACHE	NBP2-22449	Novus Biologicals	Human, Mouse, Rat +	Mono	WB, ICC, IHC, FC, GS, IP	No
Choline Acetyltransferase/ChAT	NBP1-30052	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC	No
Choline Acetyltransferase/ChAT	AF3447	R&D Systems	Human	Poly	WB, IHC	Yes
VAchT/SLC18A3	NB110-74764	Novus Biologicals	Human, Mouse, Rat	Poly	WB, IHC	No

Markers for Motor Neurons

Marker	Catalog #	Brand	Species	Clonality	Applications	Conjugate
Islet-1	NBP2-33831	Novus Biologicals	Human, Mouse, Rat	Poly	ICC, IHC	No
Islet-1	AF1837	R&D Systems	Human	Poly	WB, ICC	Yes
Islet-2	AF4244	R&D Systems	Human	Poly	WB, IHC	Yes
Neurogenin-2	MAB3314	R&D Systems	Human, Rat	Mono	IHC	No
Olig2	NBP1-28667	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC, IP	No
Olig2	AF2418	R&D Systems	Human	Poly	WB, ICC, IHC, ChIP	Yes



VGLUT2 in Human Brain. The Vesicular Glutamate Transporter 2 (VGLUT2) was detected in immersion-fixed paraffin-embedded sections of human brain (cerebellum) using a Mouse Anti-Human/Mouse/Rat VGLUT2 Monoclonal Antibody (Novus Biologicals, Catalog # NBP2-46641). The cerebellar tissue was stained using HRP and DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to glutamatergic synapses in the molecular and granular layers of the cerebellum.



$GABA_B$ R1 in Rat DRG. $GABA_B$ R1 was detected in perfusion-fixed frozen sections of rat DRG dorsal root ganglia (DRG) using a Sheep Anti-Mouse/Rat $GABA_B$ R1 Antigen Affinity-Purified Polyclonal Antibody (Catalog # AF7000). The tissue was stained using the NorthernLights™ 557-Conjugated Donkey Anti-Sheep IgG Secondary Antibody (Catalog # NL010; red) and counterstained with DAPI (blue). Specific staining was localized to the cell bodies of DRG neurons. All cited reagents are from R&D Systems.

Antibodies, continued

Microglia Markers - Steady State

Marker	Catalog #	Brand	Species	Clonality	Applications	Conjugate
AIF-1/Iba1	NB100-1028	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, PEP-ELISA	No
AIF-1/Iba1	MAB7308	R&D Systems	Human	Mono	IHC	No
CD11b/Integrin αM	NB110-89474	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, FC, SW	Yes
CD11b/Integrin αM	MAB1124	R&D Systems	Mouse	Mono	ICC, IHC, FC, IP	Yes
CD45 (low expression)	NB100-77417	Novus Biologicals	Mouse	Mono	WB, ICC, IHC, FA, FC, IP, IV	Yes
CX3CR1	NBP1-76949	Novus Biologicals	Human, Mouse, Rat	Poly	ICC, IHC, ELISA, FC	No
F4/80	NB600-404	Novus Biologicals	Mouse	Mono	WB, ICC, IHC, EM, FC, IP, RI	Yes
F4/80	MAB5580	R&D Systems	Mouse	Mono	ICC, IHC, FC	Yes
Glut5	NBP1-47980	Novus Biologicals	Human +	Mono	WB, IHC, FC, IP	No
M-CSF R/CD115	AF3818	R&D Systems	Mouse	Poly	ICC, WB	Yes
Mer	AF591	R&D Systems	Mouse	Poly	WB, FC	Yes
TMEM119	NBP2-30551	Novus Biologicals	Human	Poly	IHC	No
TROY/TNFRSF19	AF723	R&D Systems	Mouse	Poly	WB, IHC, ELISA	No

Astrocyte Markers

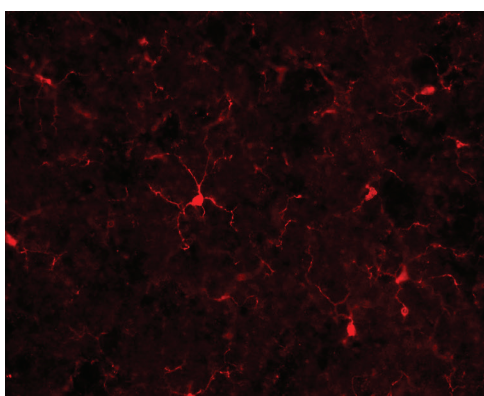
Marker	Catalog #	Brand	Species	Clonality	Applications	Conjugate
A2B5	MAB1416	R&D Systems	Human, Mouse, Rat +	Mono	ICC, FC	Yes
ALDH1L1	NBP2-25143	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC	No
Aldolase C	NBP1-90954	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC	No
Astrocytomas (Clone J1–31)	NBP2-29820	Novus Biologicals	Human, Rat	Mono	WB, ICC, IHC	No
Aquaporin-4	NBP1-87679	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
CD44	AF6127	R&D Systems	Mouse, Rat +	Poly	WB, ICC, B/N, FC	No
Connexin 43/GJA1	NB300-309	Novus Biologicals	Rat +	Poly	WB	No
EAAT1/GLAST-1/SLC1A3	NB100-1869	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, ELISA, FC	Yes
EAAT2/GLT1	NBP1-20136	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC, FC, IV	No
FGF R3	NBP2-52469	Novus Biologicals	Human	Mono	WB, ICC, IHC, ELISA, FC	No
Galectin-3	AF1197	R&D Systems	Mouse	Poly	WB, IHC, SW	Yes
GFAP	NB300-141	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, SW	No
GFAP	AF2594	R&D Systems	Human, Rat	Poly	WB, ICC, SW	Yes
Glutamine Synthetase	NB110-41404	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, IHC	No
HES-1 (Hairy 1)	NBP1-30912	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IP	No
Musashi-1	NB100-1759	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
Neuroligin 3	NBP1-90080	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	No
Notch-1	AF1057	R&D Systems	Mouse, Rat	Poly	WB, ICC, IHC, B/N, FC	No
PEA-15	AF5588	R&D Systems	Human, Mouse	Poly	WB, IHC	No
S100B	NBP2-45224	Novus Biologicals	Human, Mouse, Rat +	Mono	WB, ICC, IHC, FC	Yes
Survivin	NB500-201	Novus Biologicals	Human, Mouse, Rat +	Poly	WB, ICC, IHC, ChIP, ELISA, FC, IP, SW	Yes
Survivn	AF886	R&D Systems	Human	Poly	WB, IHC, SW	No
Thrombospondin-1	NB100-2059	Novus Biologicals	Human, Mouse, Rat +	Mono	WB, ICC, IHC, B/N, IP	No
Thrombospondin-1	AF3074	R&D Systems	Human	Poly	WB, IHC, SW	Yes

Species Key: + Additional Species Available

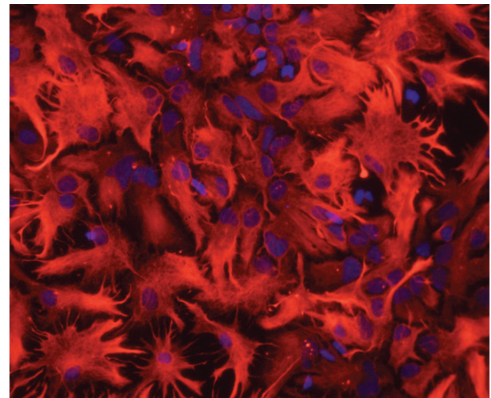
Applications Key: B/N (Blocking/Neutralization), ChIP (Chromatin Immunoprecipitation), ELISA (ELISA Capture and/or Detection), EM (Electron Microscopy), FA (Functional Assay), FC (Flow Cytometry), GS (Gel Shift), ICC (Immunocytochemistry), IHC (Immunohistochemistry), IP (Immunoprecipitation), IV (*In Vitro*), PEP-ELISA (Peptide-ELISA), RI (Radioimmunoassay), SW (Simple Western™), and WB (Western blot)

Oligodendrocyte Markers

Marker	Catalog #	Brand	Species	Clonality	Applications	Conjugate
APC	NB100-91662	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC	Yes
Caspr2	NBP1-49575	Novus Biologicals	Human, Mouse, Rat	Mono	WB, IHC, IP	No
Claudin-11	NBP1-82470	Novus Biologicals	Human, Mouse	Poly	IHC	No
CNPase	NBP2-46617	Novus Biologicals	Human, Mouse, Rat	Mono	WB, IHC	No
EZH2	AF4767	R&D Systems	Human, Mouse	Poly	WB, ICC	No
Galactosylceramidase/GALC	NBP2-16578	Novus Biologicals	Human, Rat +	Poly	WB, IHC	No
MAG/Siglec-4a	AF538	R&D Systems	Rat	Poly	WB, IHC	Yes
MBP	NB110-79873	Novus Biologicals	Human, Mouse, Rat	Poly	WB, ICC, IHC, ELISA, SW	Yes
MBP	MAB42282	R&D Systems	Human, Mouse, Rat	Mono	WB, ICC	No
MOG	NBP2-46634	Novus Biologicals	Human, Mouse, Rat	Mono	WB, IHC	No
Myelin PLP	NBP1-87781	Novus Biologicals	Human, Mouse, Rat	Poly	WB, IHC	No
NG2/MCSP	NB100-2688	Novus Biologicals	Human	Mono	WB, ICC, IHC, FC, IP	Yes
NG2/MCSP	MAB2585	R&D Systems	Human	Mono	WB, IHC, FC	Yes
NKX2.2	NBP2-29432	Novus Biologicals	Human, Mouse, Rat +	Mono	ICC, IHC, FC	No
Olig1	MAB2417	R&D Systems	Human, Mouse	Mono	WB, IHC	No
Olig2	AF2418	R&D Systems	Human	Poly	WB, ICC, IHC, ChIP	Yes
Oligodendrocyte Marker O1	MAB1327	R&D Systems	Human, Mouse, Rat +	Mono	ICC, FC	No
Oligodendrocyte Marker O4	MAB1326	R&D Systems	Human, Mouse, Rat +	Mono	ICC, FC	Yes
OMgp	NBP1-82483	Novus Biologicals	Human, Mouse, Rat	Poly	WB, IHC	No
PDGF Rα	NBP1-44581	Novus Biologicals	Human	Mono	ICC, FC	Yes
PDGF Rα	AF1062	R&D Systems	Mouse	Poly	WB, IHC, B/N	Yes
SOX10	MAB2864	R&D Systems	Human, Rat +	Mono	ICC, IHC	Yes



AIF-1/Iba1 in Mouse Spinal Cord. AIF-1/Iba1 was detected in perfusion-fixed sections of mouse spinal cord using a Goat Anti-Human/Mouse/Rat Anti-AIF-1/Iba1 Polyclonal Antibody (Novus Biologicals, Catalog # NB100-1028). The tissue was stained using an Alexa Fluor® 555-conjugated donkey anti-goat IgG secondary antibody (red).



GFAP in Rat Astrocytes. Glial Fibrillary Acidic Protein (GFAP) was detected in immersion-fixed rat astrocytes using a Sheep Anti-Human GFAP Antigen Affinity-Purified Polyclonal Antibody (Catalog # AF2594). The cells were stained using the NorthernLights™ 557-Conjugated Donkey Anti-Sheep IgG Secondary Antibody (Catalog # NL010; red) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. All cited reagents are from R&D Systems.

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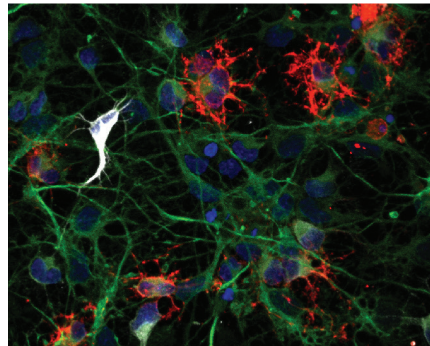
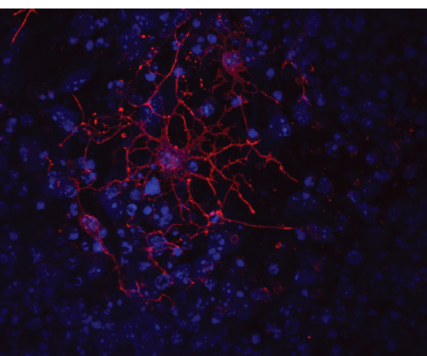
Identification and Verification Kits



βIII Tubulin, GFAP, and Oligodendrocyte Marker O4 in Differentiated Rat Cortical Stem Cells. Rat cortical stem cells (Catalog # NSC001) were differentiated into neurons, astrocytes, and oligodendrocytes using the Human/Mouse/Rat Neural Lineage Functional Identification Kit (Catalog # SC028). Differentiated cells were stained with cell-specific antibodies from the Human/Mouse/Rat Neural 3-Color Immunocytochemistry Kit (Catalog # SC024). Neurons were stained with a NL637-Conjugated Mouse Anti-Neuron-Specific βIII Tubulin Monoclonal Antibody (Clone TuJ-1; white), astrocytes were stained with a NL493-Conjugated Sheep Anti-GFAP Antigen Affinity-Purified Polyclonal Antibody (green), and oligodendrocytes were stained with a NL557-Conjugated Mouse Anti-Oligodendrocyte Marker O4 Monoclonal Antibody (red). The nuclei were counterstained with DAPI (blue). All cited reagents are from R&D Systems.

Differentiation Kits



Mouse Oligodendrocytes Generated Using the Oligodendrocyte Differentiation Kit. D3 mouse embryonic stem cells were expanded in KO-ES media and differentiated into oligodendrocytes using the Mouse Oligodendrocyte Differentiation Kit (Catalog # SC004). An oligodendrocyte phenotype was detected using a Mouse Anti-Human/Mouse/Rat/Chicken Oligodendrocyte Marker O4 Monoclonal Antibody (Catalog # MAB1326) followed by staining with the NL557-Conjugated Goat Anti-Mouse IgM Secondary Antibody (Catalog # NL019; red). The nuclei were counterstained with DAPI (blue). All cited reagents are from R&D Systems.

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R&D Systems® Cortical Stem Cells and Cryopreservation Medium

Product	Catalog #	Description
Rat Primary Cortical Stem Cells	NSC001	Isolated from E14.5 Sprague-Dawley rats; 3×10^6 cells/vial
Mouse Primary Cortical Stem Cells	NSC002	Isolated from E14.5 CD-1 mice; 2×10^6 cells/vial
CryoDefend™-Stem Cells	CCM018	Media for protein-free cryopreservation of stem cells

R&D Systems® Neural Stem Cell Kits

Product	Catalog #	Description	Species
Neural Lineage Functional Identification Kit	SC028	Verify multipotency of human, mouse, and rat neural progenitor cells (NPCs) by functional differentiation	Human, Mouse, Rat
Neural 3-Color Immunocytochemistry Kit	SC024	Characterize human, mouse, and rat neural cells differentiated from NPCs	Human, Mouse, Rat
Neural Progenitor Cell Marker Antibody Panel	SC025	Contains a collection of neural progenitor markers to verify multipotency	Human, Mouse, Rat
Dopaminergic Neuron Differentiation Kit	SC001B	Differentiates human and mouse ES/iPS cells into dopaminergic neurons under serum-free conditions	Human, Mouse
Oligodendrocyte Differentiaion Kit	SC004	Differentiates mouse ES/iPS cells into oligodendrocytes under serum-free conditions	Mouse

R&D Systems® Proteins for Neural Stem Cell Research

Molecule	Catalog #	Species	Source
BDNF	248-BD	Human, Mouse, Rat, Canine, Equine	*Sf* 21
BMP-2	355-BM	Human	CHO
BMP-4	314-BP	Human	NS0
BMP-4, GMP	314-GMP	Human	NS0
BMP-4, ProDots®	PRD314	Human	NS0
BMP-4	5020-BP	Mouse	CHO
CNTF	257-NT	Human	*E. coli*
CNTF	557-NT	Rat	*E. coli*
EGF	236-EG	Human	*E. coli*
EGF, GMP	236-GMP	Human	*E. coli*
EGF, ProDots	PRD236	Human	*E. coli*
EGF	2028-EG	Mouse	*E. coli*
EGF	3214-EG	Rat	*E. coli*
FGF basic	233-FB	Human	*E. coli*
FGF basic, GMP	233-GMP	Human	*E. coli*
FGF basic, ProDots	PRD233	Human	*E. coli*
FGF basic	3139-FB	Mouse	*E. coli*
FGF basic	3339-FB	Rat	*E. coli*
FGF-8b	423-F8	Human, Mouse	*E. coli*
FGF-10	345-FG	Human	*E. coli*
FGF-10, ProDots	PRD345	Human	*E. coli*
FGF-10	6224-FG	Mouse	*E. coli*
FGF-10	7804-FG	Rat	*E. coli*
GDNF	212-GD	Human	NS0
GDNF	512-GF	Rat	*Sf* 21
IGF-I	291-G1	Human	*E. coli*
IGF-I, GMP	291-GMP	Human	*E. coli*
IGF-I, ProDots	PRD291	Human	*E. coli*

Molecule	Catalog #	Species	Source
IGF-I	791-MG	Mouse	*E. coli*
IGF-I	4326-RG	Rat	*E. coli*
β-NGF	256-GF	Human	NS0
β-NGF	1156-NG	Mouse	NS0
β-NGF	556-NG	Rat	*Sf* 21
NT-3	267-N3	Human	*Sf* 21
NT-4	268-N4	Human	*Sf* 21
NT-4	3236-N4	Mouse	NS0
PDGF	120-HD	Human	Human Platelets
PDGF-AA	221-AA	Human	*E. coli*
PDGF-AA	1055-AA	Rat	*E. coli*
PDGF-AB	222-AB	Human	*E. coli*
PDGF-AB	1115-AB	Rat	*E. coli*
PDGF-BB	220-BB	Human	*E. coli*
PDGF-BB, GMP	220-GMP	Human	*E. coli*
PDGF-BB, ProDots	PRD220	Human	*E. coli*
PDGF-BB	520-BB	Rat	*E. coli*
Sonic Hedgehog (High Activity)	8908-SH	Human	HEK293
Sonic Hedgehog (C24II, N-Terminus)	1845-SH	Human	*E. coli*
Sonic Hedgehog (C24II, N-Terminus), GMP	1845-GMP	Human	*E. coli*
Sonic Hedgehog (C25II, N-Terminus)	464-SH	Mouse	*E. coli*
VEGF 164	493-MV	Mouse	*Sf* 21
VEGF 164	564-RV	Rat	NS0
VEGF 165	293-VE	Human	*Sf* 21
VEGF 165, ProDots	PRD293	Human	*Sf* 21

Source Key: CHO (Chinese hamster ovary cell line), HEK293 (Human embryonic kidney cell line), NS0 (Mouse myeloma cell line), *Sf* (*Spodoptera frugiperda*)

Neural Stem Cell Products, continued

Tocris® Small Molecules for Neural Stem Cell Expansion

Product	Catalog #	Primary Action
17-AAG	1515	Selective HSP90 inhibitor; protects neural progenitors from naturally occuring apoptosis
Amiodarone	4095	Ion channel blocker; selectively inhibits NSC proliferation in hESC-derived cell populations
CHIR 99021	4423	Selective GSK-3 inhibitor; enhances ESC self-renewal in combination with PD 0325901 (Tocris, Catalog # 4192)
Cyclopamine	1623	Hedgehog signaling inhibitor; suppresses the proliferation of Ptch1+/– medulloblastoma precursor cells
DMH-1	4126	Selective ALK2 Receptor inhibitor; promotes iPSC neurogenesis in combination with SB 431542 (Tocris, Catalog # 1614)
INDY	4997	Dyrk1A/B inhibitor; impairs the self-renewal capacity of NSCs
P7C3	4076	Neuroprotective and proneurogenic compound; orally available
SB 431542	1614	Induces proliferation, differentiation and sheet formation of ESC-derived endothelial cells
SU 5402	3300	Potent FGF R and VEGF R inhibitor; attenuates Integrin β4-mediated differentiation of NSCs
Y-27632	1254	Selective p160ROCK inhibitor; enhances survival of hES cells undergoing cryopreservation

Tocris® Small Molecules for Neural Stem Cell Differentiation

Product	Catalog #	Primary Action
DAPT	2634	γ-Secretase inhibitor; induces neuronal differentiation of neural cells
Fluoxetine	0927	5-HT re-uptake inhibitor; induces differentiation of neuronal precursors
Forskolin	1099	Adenylyl Cyclase activator; induces neuronal differentiation in NSCs
Isotretinoin	5513	Inducer of neuronal differentiation; endogenous agonist for retinoic acid receptors
ISX 9	4439	Induces neuronal differentiation of SVZ progenitors
KHS 101	4888	Selective inducer of neuronal differentiation in hippocampal neural progenitors
Metformin	2864	Antidiabetic agent; promotes neurogenesis
Neurodazine	3656	Induces neurogenesis in mature skeletal muscle cells
Neuropathiazol	5186	Selective inducer of neuronal differentiation in hippocampal neural progenitors
1-Oleoyl lysophosphatidic acid sodium salt	3854	Endogenous agonist of LPA1 and LPA2; inhibits differentiation of NSCs into neurons
O-Phospho-L-serine	0238	Group III mGlu agonist; inhibits proliferation and enhances neuronal differentiation of progenitor cells
Retinoic acid	0695	Retinoic Acid Receptor agonist; promotes differentiation of ESCs into neurons, glia and adipocytes
TCS 2210	3877	Inducer of neuronal differentiation in MSCs
TWS 119	3835	GSK-3β inhibitor; induces neuronal differentiation in ESCs
Valproic acid, sodium salt	2815	Histone Deacetylase inhibitor; promotes neuronal differentiation

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