NeuroPure™

E18 Primary Mouse Cortical Cells, Cat. # N200201

Genlantis
A division of Gene Therapy Systems, Inc.

Description/Content	Amount
Day 18 Embryonic C57 Mouse Cortical Cells	1 vial x 2 ml (~1 x 10^6 cells*)
NeuroPure™ Plating Medium	1 vial x 12 ml
NeuroPrep™ Medium	1 vial x 2.5 ml
NeuroPapain™ Enzyme	1 vial x 5 mg
Shipping and Storage:	NeuroPure™ Primary Mouse Cortical Cells are shipped refrigerated. Cells are stable for up to 7 days when stored at 4-8ºC. However, WE HIGHLY RECOMMEND PLATING WTHIN 1 TO 2 DAYS FOR BEST RESULTS.

Related Products	Amount	Catalog #
NeuroPure™ Primary E18 Mouse Hippocampal Cells	~1 x 10^5 cells*	N100201
NeuroPure™ Primary E18 Mouse Hypothalamus Cells	1 Hypothalam	N400201
NeuroPure™ Primary E18 Mouse Striatum Cells	1 Striatum	N500201
NeuroPure™ Primary E18 Mouse Spinal Cord Cells	1 Spinal Cord	N600201
NeuroPure™ Primary E18 Mouse Midbrain Cells	1 Midbrain	N700201
NeuroPure™ Primary E18 Mouse Hippocampal Astrocytes	~1 x 10^5 cells*	N800201
NeuroPure™ Primary E18 Mouse Cortical Astrocytes	~1 x 10^6 cells*	N900201
NeuroPrep™ Medium	1 x 100 ml	NM100100
NeuroPapain™ Enzyme	1 x 100 mg	NM100200
NeuroFECT™ Transfection Reagent	75 - 300 Rxns	T800075
GeneSilencer® siRNA Transfection Reagent	200 Rxns.	T500750

*Cell numbers may vary by + or – 50%

INTRODUCTION

NeuroPure™ E18 Primary Mouse Cortical Cells are live neurons provided as micro-surgically dissected regions of day 18 embryonic C57 mouse brain. These cells are prepared fresh each week and shipped in a nutrient rich medium that keeps them alive for up to 14 days under refrigeration. Following simple dissociation steps, the NeuroPure cells can be quickly plated on almost any poly-lysine coated substrate using the provided NeuroPure Plating Medium. NeuroPure cells are ideal for a wide variety of applications including: transfection, pharmacology, electrophysiology, immunocytochemistry, and neuronal development studies.

MATERIALS AND METHODS

I. Preparation for Culturing

1. Culture plates or coverslips may be prepared by coating with poly-D-lysine (0.15 ml/cm^2, 50 µg/ml, 135 kD, (Sigma #P6407) for 1-20 hr. Rinse once with 18 Mohm diH_20, and let dry.

   Alternatively, BD PureCoat™ poly-amine coated culture plates (BD Biosciences) may be used to reduce formation of neurospheres.

NOTES:
- If using *poly-D- lysine (PDL) coated substrate, we recommend that you prepare the coated substrate yourself, rather than purchasing pre-coated PDL substrates.*
- *If NeuroPure cells will be cultured for >3-4 days, we recommend using the culture medium described in Appendix A*

2. Clean the Biological Safety Cabinet with 70% alcohol to ensure it is sterile.
3. Turn the Biological Safety Cabinet blower on for 10 min. before cell culture work.
4. Make sure all serological pipettes, pipette tips, and reagent solutions are sterile.
5. Follow the standard sterilization technique and safety rules:
   a. Do not pipette with mouth.
   b. Always wear gloves and safety glasses when working with animal cells.
   c. Handle all cell culture work in a sterile hood.

II. Enzymatic Pretreatment (Optional)

Enzymatic treatment of NeuroPure tissue prior to mechanical dissociation can increase the number of viable cells by up to 100%. However, please note that for assays performed within 4 days of plating, some digestion of surface proteins is inevitable.

1. Add 5 mg of NeuroPapain Enzyme into 2.5 ml of NeuroPrep Medium. Mix at 37ºC for 15 min. to completely dissolve the NeuroPapain. Sterilize this solution with a 0.2 µm filter prior to utilizing for tissue digestion. Use within 3 hrs for best results.
2. Prior to enzymatic treatment, allow the NeuroPure tissue to settle for 15 minutes at 4ºC. Alternatively, place the tube containing the tissue in a 50 ml tube and spin down the cells at 1,100 rpm (200xg) for 1 minute. Transfer the medium from the NeuroPure cell vial to a separate sterile tube while being careful not to remove any loose tissue pieces. *Save the medium for trituration following NeuroPapain treatment.*
3. Immediately add 2 ml of sterile NeuroPapain solution to the tissue-containing tube, and allow the neuronal tissue to incubate for 30 minutes at 30 ºC. Swirl every 2 min. by hand.
4. Following incubation, spin down the cells at 1,100 rpm (200xg) for 1 minute. Remove the NeuroPapain solution, again being careful not to disturb or remove the tissue.
5. Add 1 ml of shipping medium back to the NeuroPure cells. Save the other 1 ml of shipping medium for Step 4 below.
6. Proceed to Step 3 below.

III. Preparation of Isolated Neurons

1. After receiving the cells, let them settle at 4°C for 15 minutes, OR spin down at 1,100 rpm (200xg) for 1 min.
2. Transfer 1 ml of medium from the cells tube into a sterile 50 ml screw cap tube; be careful not to disturb or remove cells from the original cells tube.
3. Using a P-1000 pipettor with a sterile blue 1 ml plastic tip (0.8-1.0 mm diameter opening) or a silanized 9-inch Pasteur

pipette with the tip fire polished until it is smooth and has a 0.8-1.0 mm diameter opening, gently pipette the cells with the medium up and down into the same container. Take care not to create bubbles. Repeat this tituration step ~15 times or until most all the cells are dispersed.

4. Transfer the dispersed cells into the 50ml tube containing the 1 ml of media from Step 2. Gently mix the cells by swirling.
5. Spin the cells at 1,100 rpm (200xg) for 1 min. Discard the supernatant while being careful not to remove any of the cells from the cell pellet.
6. Flick the tube a few times to loosen the cell pellet. Resuspend the pellet in 1 ml of the provided NeuroPure Plating Medium. Resuspend the cells by gently pipetting up and down.
7. Aliquot 20 µl and mix with 20 µl of 0.4% trypan blue.
8. Count cells with a hemocytometer and determine percentage of live cells. The expected viability is >90% with NeuroPapain treatment and ~50% without NeuroPapain treatment.
9. Further dilute the cells with NeuroPure™ Plating Medium to the desired plating density. We recommend 32 x 10^3 cells/2 cm^2 in 0.4 ml/2 cm^2 of substrate.

NOTE: *We do not recommend using antibiotics, such as Pen-Strep. They have been shown to activate epileptiform bursting activity in neurons. Nevertheless, we sometimes start our cultures in gentamicin (10 ug/ml) and rinse it away after 1 hour once the cells adhere.*

10. Incubate the cells at 37ºC with 5% CO_2 and/or 9% or 20% O_2.
11. After 4 days or longer, neurons are well differentiated. If further culture is desired, change half of medium with fresh, warm culture medium (See Appendix A).

IV. Viability Assay (Optional)

Trypan blue provides a rough estimate of cell viability, which is sufficient for many applications. However, if a more accurate quantitation of viability is desired, use the following assay

1. Rinse cells twice with PBS.
2. From an acetone stock of 15 mg/ml fluorescein diacetate (Sigma), add 15 µl (1:100 dilution of the stock) into 1.5 ml Hank's Buffered Salt Solution (HBSS). From an aqueous stock of 4.6 ml/ml propidium iodide, add 15 µl of the stock into the same 1.5 ml HBSS (1:100 dilution). Add 40 µl of that dilution to each well with 0.4 ml HBSS (further 1:100 dilution).
3. After approximately 1 minute, count using Nikon B1A filter or other blue excitation appropriate for fluorescein fluorescence. Green cells are alive. Small red nuclear stain indicates a dead cell. If desired, fix and stain with 0.25% Coomassie blue R in ethanol/acetic acid/H_20 (45/10/45), 1 min., rinse with 10% acetic acid, aspirate and dry.

APPENDICES

Appendix A: Culture Medium

For culturing NeuroPure cells for >3-4 days, we recommend the following components from Invitrogen Corporation:

Neurobasal™ Medium: Cat. # 21103; B27 Serum-Free Supplement: Cat. # 17504; Glutamax™ Cat. # 35050*.

*Neurobasal™ Medium and Glutamax™ are trademarks of Invitrogen Corporation.

Appendix B: Induction of Neuron Cell Division

By addition of Fibroblast Growth Factor 2 (Basic) (FGF2/bFGF) at a concentration of 5 ng/ml, NeuroPure cells will multiply as long as the density is kept below 240 cells/mm^2. To passage the cells, we recommend using NeuroPapain at 2 mg/ml in NeuroPrep Medium to incubate the cells for ~ 5 min at 37 ºC. For example:

a. Rinse the cells with warm NeuroPrep Medium.
b. Add 2 mg/ml NeuroPapain in NeuroPrep Medium and incubate ~5 minutes at 37ºC.
c. Carefully remove NeuroPapain and replace with the culture medium from Appendix A. Spin to collect cells.

Appendix C: Common Questions (Also, please see our NeuroPure FAQ's at http://www.genlantis.com/)

Question	Answer
How many astrocytes do the NeuroPure neuronal cells contain?	After 72 hours of culturing, we have measured <1% astrocytes. However, higher percentages of astrocytes will eventually be generated after several weeks in culture.
Do you have a detailed protocol for coating of substrates with poly-D-lysine?	Yes, Please see the Hot Files box at the bottom right corner of the NeuroPure web page in the Cells and Media section of www.genlantis.com.
How long does it take for the NeuroPure Cells to grow neurites?	Typically, the neurites become visible beneath a microscope within 48-72 hours after plating.
Which of your transfection reagents do you recommend for transfecting the NeuroPure cells?	For plasmid transfection, we recommend the NeuroFECT™ Transfection Reagent. For siRNA transfection, we recommend our GeneSilencer® siRNA Transfection Reagent.

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