NeuroPure™

Frozen E18 Rat Hippocampal Neurons, AMS.N100200

Description/Content	Amount
Frozen Day 18 Embryonic Sprague/Dawley Rat Hippocampal Neurons, Cat. No. AMS.N100200F1	1 vial (1 x 10^6 cells)
Frozen Day 18 Embryonic Sprague/Dawley Rat Hippocampal Neurons, Cat. No. AMS.N100200F4	4 vials (4 x 10^6 cells)
Shipping and Storage:	Frozen E18 Primary Rat Neurons are shipped on dry ice. Store at -70C or below upon receipt.

Related Products	Amount	Catalog #
Frozen Day 18 Embryonic Sprague/Dawley Rat Cortical Neurons	1 x 10^6 cells 4 x 10^6 cells	N200200F1 N200200F4
NeuroPure™ Primary E18 Hippocampal Cells	~1 x 10^6 cells*	N100200
NeuroPure™ Primary P8 Cerebellar Cells	~4 x 10^7 cells*	N300200
NeuroPure™ Primary E18 Hypothalamus Cells	1 pair	N400200
NeuroPure™ Primary E18 Striatum Cells	~1 x 10^6 cells*	N500200
NeuroPure™ Primary E18 Spinal Cord Cells	~1 x 10^6 cells*	N600200
NeuroPure™ Primary E18 Midbrain Cells	1 pair	N700200
NeuroPrep™ Medium	1 x 100 ml	NM100100
NeuroPapain™ Enzyme	1 x 100 mg	NM100200
NeuroFECT™ Transfection Reagent	75 - 300 Rxns	T800075

INTRODUCTION

NeuroPure™ Frozen E18 Primary Rat Hippocampus Cells are prepared using proprietary technology that provides a superior frozen neuron product in terms of survival and number of live cells. NeuroPure™ Frozen E18 Primary Rat Hippocampus Cells are guaranteed to produce 20% survival after thawing and 4 days in culture. 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. Prepare culture plate or coverslips by coating with poly-D-lysine (0.15 ml/cm^2, 100 µg/ml, 135 kD, Sigma Cat. #P6407) for 1-20 hr. Rinse one time with 18 Mohm diH_20, and let dry.

NOTES: • *We strongly recommend that you prepare your own poly-D-lysine (PDL) coated substrate, rather than purchasing pre-coated PDL substrates.*

2. Prepare culture medium (see Appendix A), bring to room temperature.
3. Clean the Biological Safety Cabinet with 70% alcohol to ensure it is sterile.
4. Turn the Biological Safety Cabinet blower on for 10 min. before cell culture work.
5. Make sure all serological pipettes, pipette tips, and reagent solutions are sterile.
6. 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. Thawing and Plating Neurons

1. Thaw the cells by placing the tube in a 37ºC waterbath (no more than 3 min). Remove immediately after the cells are thawed. Wipe the vial with 70% ethanol. Do not vortex or shake the tube.
2. Transfer 0.21 ml to a 50 ml centrifuge tube being careful not to create bubbles.
3. Add 1.91 ml of pre-warmed room temperature medium (see Appendix A) drop-by-drop over a period of 30 sec. Mix gently as you add the medium. Mix by inversion two times.
4. Immediately plate the cells at 0.2 ml/cm^2 of substrate (range 0.15-0.5 ml/cm^2) or 2 ml per dish containing 3 pre-coated cover slips. Mix cells gently every 2 min in between aliquots.
5. Incubate plated cells for 1-3 h at 37ºC, 5% CO2, 9% O2 (or ambient oxygen). Place more medium in the incubator with loose cap to equilibrate the pH for step 8.
6. Mix leftover cells from step 4 and aliquot 20 µl and mix with 20 µl of 0.4% trypan blue.
7. Count cells with a hemocytometer and determine percentage of live cells.
8. Perform medium exchange after the initial 1-3 h incubation. Drain and aspirate medium over cells and immediately add fresh equilibrated medium from step 5 (0.2-0.4 ml/cm^2 of substrate, use 2 ml for 35 mm dish). Do not let the cells dry out. Add medium gently from the side so that not to disturb the adherent cells. Observe bright phase adherent cells to ensure attachment to substrate.
9. Incubate the cells at 37ºC with 5% CO_2 and/or 9% or 20% O_2.
10. After 4 days or longer, neurons are ready for your use. If further culture is desired, change half of medium with fresh, warm culture medium (See Appendix A). One-half medium should be exchanged every 3-4 days.
11. Measure viability by counting live, phase-bright cells with processes. Small, bright cells are dead or apoptosing.

III. 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, the following assay may be used.

1. Rinse cells twice with PBS or HBSS.
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 (Sigma), 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 1:10 dilution).
3. After approximately 1 minute, count using Nikon B1A, G1B filters 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 frozen cells, we recommend the following components from Invitrogen Corporation:

Neurobasal™ Medium, Cat. # 21103-049; 2% B27 Serum-Free Supplement, Cat. # 17504-044; 0.5 mM Glutamax™, Cat. # 35050-061*

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