Single Embryonic Mouse Primary Cortical Neuron Culture--Dissociated via Trypsin

Day 1

Poly-D-Lysine coating:

1.) Add 333.35mL of sterile ddH_2O to 50mg of PDL
2.) Mix and filter thru 0.22 μm filter.
3). Aliquot and store @ -20°C
  a.) Use within 1-2 weeks
  b.) Only freeze / thaw once…

To coat plates:

1). Thaw PDL at 37°C
2). Filter thru 0.22 μm filter
3). Plate and incubate at 37°C overnight

Plating volumes:

96 well plates	50-60 μL/well.
4 well slides	300 μL/well
24 well plates	500 μL/well
6 well plates	2 mL/well
T25 flasks	4 mL/flask

Day 2

Wash / Dry Poly-D-Lysine Coated Plates

1.) Vacuum off PDL and wash twice with sterile ddH_2O
2.) After the second wash is removed, allow the plates to dry completely (uncovered in the hood)

Prepare Mouse Laminin Substrate:

1.) Add 40 mL of base media to 1.1 mg of mouse laminin
  * Do not filter *
2.) Coat PDL plates with laminin (same volumes as for PDL) and place in incubator overnight

Day 3

Wash Laminated plates

1.) Vacuum off laminin and wash twice with an equal volume of base media.
2.) After the second wash is removed, replace a third time with base media and place in incubator until time of plating.

Dissection / Preparation:

1.) Warm dissection media (HBSS w/o calcium or magnesium) and place in appropriate dishes.
2.) Warm plating media in 37°C H_2O bath.
3.) Sacrifice the mother by cervical dislocation.
4.) Spray lower abdomen with 70% EtOH and cut through skin and muscle with a pair of scissors exposing the uterus, intestines and embryos.
5.) Cut embryos from amniotic sac.
6.) Remove brain from skull.
7.) Dissect the cortex from each lobe.
8.) Using a spring scissors, cut each cortex into about 3 pieces
9.) Pick up the cortical pieces along with 1 mL of the HBSS using a blue tip and place into a 15 mL conical tube.

10.) Add 100 uL of 0.25% Trypsin to the 1 mL of dissection medium

Final trypsin concentration = 0.025%

11.) Place tissue / enzyme in 37°C incubator for 30 minutes.

12.) After the 30 minute digestion is complete, spin the tissue at 1500 rpm for 3 minutes.

13.) Remove the supernantant and re-suspend in 1 mL of plating media.

14.) Count cells on hemacytometer and dilute to 0.5 $x10^6$ cells/mL

Feeding / Inhibition:

- On DIV5, remove 50% of the media and replace with fresh plating media along with Ara-C at a final concentration of 1 μM.
    - Aliquots of 10 mM AraC in ddH_2O are stored @ -20°C
    - 20 μl of 10 mM AraC is added to 100mL of feeding media = 2 μM
    - 1/2 change of media = final of **1μM**.
        - Note: Never do a 100% media change.
- After the DIV5 inhibitory feeding, feed the cells every 3 days (without Ara-C) using the 50% exchange method and replacing with feeding media (plating media but without serum, 2% B27 is supplemented instead).

Solutions:

Neurobasal Plating Media

1.) Defrost cFBS and HS in 37°C H_2O bath.

2.) From 500 mL Neurobasal bottle, remove 50 mL.

3.) Add 25 mL of cFBS and 25 mL of HS

4.) Filter through 0.22 μm filter system, label and store @ 4°C

* When ready to plate / feed, add FRESH L-glutamine (200 mM stock, 2mM final) and Pen-
Strep (100% stock, 0.5 to 1% final).

NB/B27 Feeding Media

1.) Defrost B27 in 37°C H_2O bath.

2.) From 500 mL Neurobasal bottle, remove 10 mL.

3.) Add 10 mL of B27

4.) Filter through 0.22 μm filter system, label and store @ 4°C.

When ready to feed, add FRESH L-glutamine (200 mM stock, 2mM final) and Pen-Strep (100% stock, 0.5 to 1% final).

Product Ordering Information:

0.25% Trypsin, 15050-065, Invitrogen
Ara-C, C6645, Sigma
B27 Supplement, 17504044, Invitrogen
FBS (Dialyzed), SH30071.03HI, HyClone
L-glutamine, 25030-081, Invitrogen
Neurobasal Media, 21103-049, Invitrogen
NUNC 6 well plates, 140675, Fisher
Costar 24 well plates, 3526, Fisher
Poly-D-Lysine, P0899, Sigma