Dissociated Olfactory Bulb Cultures

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.

Tissue Preparation:

- Add 5 mL of HBSS to a conical tube and a few 10 mm dishes for dissecting and allow to warm to room temperature
- Warm NB/B27 (or MEM) Plating Media in 37°C water bath
- Remove an aliquot of 0.25% trypsin and an aliquot of 100 μM DNase from the –20° freezer and allow to thaw at room temperature
- Remove cFBS from the –20°C freezer and allow to thaw in water-bath
- Decapitate mouse pups and place heads in 10 mm dishes with HBSS
- Make an incision from the base of the skull upward to the front of the nose
- Remove any extra skull or skin to reveal the olfactory bulb (OB)
- Use a curved forcep to remove the OB from the head and place into the conical tube containing 5 mL of HBSS.

Plating Procedure:

- In the culture hood remove the HBSS from the tissue and add 500 μl of 0.25% Trypsin and 5μl of DNase **per bulb** to the tube. Note, this can be done with a

single brain (2 bulbs) per tube, or an entire litter of bulbs → Trypsin and DNase should be adjusted accordingly.)
- Use a blue pipette tip to break up the bulb by triturating ~5 times
- Incubate triturated OB's in trypsin/DNase mix in a 37°C waterbath for 30 minutes
- After incubation is complete, centrifuge cells at 1000 rpm and remove the Trypins/DNase mix leaving behind a cell pellet
- Add 2 mL of cFBS plating media to inactivate the trypsin (leave on for about 2 minutes)
- Centrifuge cells again at 1000 rpm and remove the cFBS, leaving behind the cell pellet
- Re-suspend the cell pellet in 1 mL of chosen plating media per every 2 OB's
- Gently triturate about 7 times with a blue pipette tip
- Gently triturate again about 7 times with a flamed glass Pasteur pipette
- Pour cell suspension through a 70μm cell strainer and into a 50 mL conical tube
- Use a flamed glass Pasteur pipette to remove the cell suspension from the 50 mL conical and place it into a 15 mL conical tube.
- Gently spin cells for 5 minutes at 1000 rpm to pellet the cells
- Remove the NB/B27 plating media from the pellet and re-suspend the cells in 1 mL of fresh NB/B27 per every 2 OB's using a blue pipette tip.

Cell Counting:

- Dilute cells for counting by adding 20 μl of the cell suspension to 180 μl of plating media (10x dilution)
- Count cells and dilute to **2.0x10^6 cells/mL** with NB/B27 plating media
- Plate cells using the following volumes:
  - 96 well plates 100 μL/well
  - 4 chamber slides 500 μL/chamber
  - 24 well plates 1 mL/well
  - 6 well plates 4 mL/well
  - T25 flasks 10 mL/flask
  - 4 well plates 500 μL/well

Feeding/Inhibition:

- Feed cells every day by removing 50% of the old media and adding back 50% fresh NB/B27 media with Ara-C
- The final concentration of Ara-C is 1 μM
  - Aliquots of 10 mM Ara-C in ddH_2O are stored @ -20°C
  - 10μl of 10 mM Ara-C is added to 50 mL of feeding media.

Solutions:

- **Neurobasal / B27 Supplement Media**
  - Defrost B27 and L-glutamine in 37°C waterbath
  - Remove 15 mL from 500 mL bottle of Neurobasal
  - Add 10 mL of B27
  - Add 5 mL of 200 mM Glutamine (2 mM final)
    - If necessary, may add 0.5-1.0% of the following:
      - Pen/strep
      - Gentamycin
      - Kanamycin
  - Filter through 0.22 micron filter system, label and store @ 4°C

Product Ordering Information:

70 micron cell strainers, 08-771-2, Becton Dickson via Fisher
Ara-C, C6645, Sigma
B27 Supplement, 17504-044, Invitrogen
DNase, D4513m Sigma
Falcon 24 well plates, 523411, Fisher
FBS (Characterized), SH30071.03, HyClone
Gentamycin, G3632, Sigma
L-glutamine, 25030-081, Invitrogen
HEPES (Hemisodium Salt), H7637, Sigma
Horse Serum, SH30074.04, HyClone
Kanamycin, K4000, Sigma
MEM, 15-010-CV, Media Tech
Neurobasal, 21103-049, Invitrogen
NUNC 6 well plates, 140675, Fisher
NUNC 96 well plates, 25382-342, Fisher
Poly-D-Lysine, P0899, Sigma
Laminin, 354232, Becton Dickson
Trypsin, T4549, Sigma