

## **Primary Human Olfactory Receptor Neuron Culture**

### **Substrate Coating**

- **Human laminin:**
  - Bring up 0.5 mg of laminin in 12.5 mL of MEM-D-Val
    - Do not filter.
    - Final concentration of 0.04 mg/mL
  - Coat culture dishes with half the volume used for plating
    - 6 well plates            2 mL
    - 4 chamber slides       250  $\mu$ l
  - If there is any laminin left, it can be aliquoted and stored at -20°C.
    - **NOTE:**
      - Laminin can only be frozen and thawed *once*.
      - Laminin *cannot* be filtered!
  - Incubate plates @ 37°C for a minimum of 2 hours (Best if left overnight)
  - Wash three times with MEM-D-Val
    - After the third wash, place the plates with MEM-D-Val back into the incubator
      - Do not let the plates dry...

### **Preparation:**

- Obtain the following items from -20°C freezer and allow to thaw in 37°C waterbath:
  - NuSerum
  - dFBS
  - Fungizone
  - DNase 0.1%
  - 10x Trypsin
  - NGF (10  $\mu$ g/mL) / BDNF (50  $\mu$ g/mL)
  - Ara-C (100 mM)
- Spin tissue @ 1,000 rpm for 5 minutes
- Mince tissue with sterile spring scissors for about 2 minutes
- Wash scissor with 5 mL of MEM-AIR
  - Total volume in conical = 10 mL
- Centrifuge @ 2,000 rpm for 10 minutes
- While centrifuging:
  - Prepare plating media as follows:
    - 80 mL of MEM-D-Val
    - 1 mL of fungizone (1% final)
    - 5 mL of NuSerum (5% final)
    - 12.5  $\mu$ L of NGF (12.5  $\mu$ g/mL final)
    - 10  $\mu$ L Ara-C (10  $\mu$ M final)
    - 2 mL Kana-glycine (2% final)
    - 1 mL Gentamycin (1% final)
    - 500  $\mu$ L of PenStrep (0.05% final)
      - Optional
    - Filter and keep in 37°C water bath until needed.
  - In hood place 4.5 mL of HBSS into a 15 mL conical tube.
  - Add 75  $\mu$ L of 10x Trypsin and 7.5  $\mu$ l of DNase
- When done spinning, remove the supernatant from the tissue.
- Add the HBSS/Trypsin/DNase mix.
- Incubate in 37°C waterbath for 22 minutes.

- Centrifuge at 1000 rpm for 2 minutes.
- Remove Trypsins/DNAase mix and wash with 5 mL of HBSS.
- Remove HBSS and wash with 5 mL of dialyzed FBS to inactivate the enzyme.
- Remove dFBS and add 5 mL of MEM-D-Val.
- Triturate about 10 times with a 10 mL pipette.
- Triturate again about 10 times with glass pipette.
- Pour through a 70µm nylon cell strainer and into a 50 mL conical tube.
- Centrifuge cell suspension for 5 minutes @ 2000 rpm.
- Remove MEM-D-Val via vacuum.
- Re-suspend in 5 mL of plating media.
- Make a 10x dilution of cells and count.
- Plate cells at a final concentration of  $2.0 \times 10^6$  cells/mL.
- Feed cells every other day by removing  $\frac{1}{2}$  of the volume and replacing with fresh feeding media (either 15% or 0.05% medias – see below).
  - **At each feeding (optional):**
    - Add final concentrations of 10 µM Ara-C and 2.5 ng/mL NGF and BDNF to the media...

#### Base Medias:

- **MEM-D-Val**
  - 9.28 g of powder media
  - 2.2 g sodium bicarbonate
  - 0.292 g L-glutamine
    - Bring up to 1 L
    - pH to 7.4
    - Filter sterile and store @ 4°C
- **MEM-AIR**
  - 1 pkg DMEM with high glucose
  - 4.8 g Hepes, Sodium Salt
    - Bring up to 1 L
    - pH to 7.4
    - Filter sterile and store @ 4°C

#### Feeding Medias:

- **15% feeding media**
  - 410 mL of MEM-D-Val
  - 75 mL of dFBS
  - 10 mL of Kanamycin
  - 5 mL of Gentamycin
  - 2.5 mL of PenStrep (0.5% final)
    - Optional
  - Filter Sterile and store @ 4° C
- **0.5% Feeding Media**
  - 480 mL of MEM-D-Val
  - 2.5 mL of dFBS
  - 10 mL of Kana-glycine
  - 5 mL of Gentamycin
  - 2.5mL of Penstrep (0.5%)
    - Optional
  - Filter sterile and store @ 4°C

**Product Ordering Information:**

Trypsin (10X), T4549, Sigma

Ara-C, C6645, Sigma

FBS (Dialyzed), 26400-044, Invitrogen

Gentamycin Sulfate, G3632, Sigma

Horse Serum, SH30074.03HI, HyClone

Kanamycin, K4000, Sigma

NGF, 100700, Roche

BDNF, AB1534, Chemicon

MEM-D-Val, 99-909-PB (Special Order), Media Tech (Cellgro)

MEM-AIR, 12100-046, Invitrogen

Pen-Strep, 15140-148, Invitrogen

Fungizone, 152900187, Invitrogen

NuSerum, 355504, Collaborative BioMed

Human Laminin, L6274, Sigma

40 micron cell strainers, 352340, Becton Dickson

70 micron cell strainers, 352350, Becton Dickson

NUNC 4 well glass slides, 62407-330, VWR

NUNC 4 well plates, 176740, VWR