

## Primary Rat Olfactory Receptor Neuron Culture--Dissociated via Enzyme Mix

### Day/Morning before prep:

1. Substrate (Laminin) coating of plates
  - a. 1.6 mg of laminin (kept at  $-80^{\circ}\text{C}$ ) is brought up in 40 mL of MEM-D-Val (Do not filter!).
    - i. Final concentration = 0.04 mg/mL
  - b. Coat plate with half the amount used for plating i.e.

2 mL	6 wells
250 $\mu\text{L}$	4 chamber slides
1 mL	12 wells
500 $\mu\text{L}$	24 wells
  - c. Incubate @  $37^{\circ}\text{C}$  for a minimum of 2 hours. Best if left over night.
  - d. Wash substrate coated plates with MEM-D-Val two times.
    - i. Do not allow plates to dry  $\rightarrow$  After second wash, add MEM-D-Val and place back in incubator until you are ready to plate cells.
2. Pre-prep:
  - a. Fill and heat circulating water bath to exactly  $37^{\circ}\text{C}$
  - b. Warm:
    - i. MEM-AIR
    - ii. MEM-D-Val
  - c. Obtain rat pups (Post-natal days 0.5 through 1)
    - i. 15 pups (one litter) = a  $\frac{1}{4}$  prep
      1. Yields roughly 16 mL @ 2 million cells / mL
    - ii. 45-50 pups = a half prep
      1. Yields roughly 55 mL @ 2 million cells / mL
    - iii. 80-100 pups = a full prep
      1. Yields roughly 130 mL @ 2 million cells / mL
3. Enzyme Digestion Mix

		<u>Half Prep</u>	<u>Full Prep</u>	<u>Storage</u>
	BSA	0.30 g	0.60 g	$4^{\circ}\text{C}$
1 mg/mL	Hyaluronidase	0.03 g	0.06 g	$-20^{\circ}\text{C}$
5 mg/mL	*Dispase	0.149 g	0.298 g	$4^{\circ}\text{C}$
250 U/mg	*Collegenase (D)	0.044 g	0.087 g	$4^{\circ}\text{C}$
3 $\mu\text{g}/\mu\text{L}$	**DNAse	35 $\mu\text{L}$	70 $\mu\text{L}$	$-20^{\circ}\text{C}$

\* Dependent on Lot

\*\* Added later

- a. Get all needed ingredients for enzymes listed above. Measure out and place in large mouth bottle with lid and stir bar.
- b. Prepare all materials needed for dissection

Scissors	Disposal bag
Curved fine forceps	Kim wipes, Paper towels
Forceps	Dissection board, pins
Large weigh boats	70% EtOH
- c. Transfer 31 mL of MEM-AIR into each of three 50mL conical tubes and keep @  $37^{\circ}\text{C}$  in heated water bath until needed.

### Dissection

1. Do dissection in batches of 5 or 10 pups... whatever you are comfortable with (consider your speed...).

2. First cut through the roof of the mouth between the nostrils and straight back to brainstem.
3. Open head into halves and pin down.
4. Remove septum from over top of the turbinate.
  - a. If cut is clean up the middle, one turbinate will be exposed and the other will be beneath the septum.
5. Remove the turbinate with fine curved forceps and place into tube containing 30 mL of MEM-AIR.
  - a. Place each half prep into a separate tube.

#### **After Dissection**

1. Spin tissue @ 1,000 RPMs for 10 minutes.
2. While spinning
  - a. Obtain the following items from  $-20^{\circ}\text{C}$  and allow to thaw in  $37^{\circ}\text{C}$  water bath:
    - i. NuSerum
    - ii. dFBS
    - iii. Fungizone
    - iv. DNase 0.1%
  - b. Prepare the enzyme digestion mix:
    - i. Add MEM-AIR to the powder enzymes
      1. 62 mL of MEM-AIR for a full prep
      2. 31 mL of MEM-AIR for a half prep
      3. 15.5 mL of MEM-AIR for a  $\frac{1}{4}$  prep
    - ii. Add DNase for a full prep...
      1. 70  $\mu\text{L}$  for a full prep
      2. 35  $\mu\text{L}$  for a half prep
      3. 17.5  $\mu\text{L}$  for a  $\frac{1}{4}$  prep
    - iii. Place in circulating water bath at  $37^{\circ}\text{C}$  with stir bar until needed.
    - iv. When ready for enzymes (after second spin - below), filter through a .22 micron filter on a 30mL syringe.
      1. Place 30 mL each into 50mL conical tubes depending on size of prep
        - a. 2 x 50 mL tubes for a full prep
        - b. 1 x 50 mL tube for a half and  $\frac{1}{4}$  preps
3. After the 10 minute spin, aspirate the supernatant (bring liquid to you to avoid vacuuming any of the pellet).
4. Transfer tissue to a 100 mm petri dish by inverting and tapping on the conical tube containing the tissue.
5. Mince the tissue with sterile curved scissors for about 2 minutes (per tube)
  - a. Ideally, you will want to cut each turbinate into *at least* two pieces...
6. Use 10 mL of MEM-AIR to wash any excess tissue from the scissor by allowing the MEM-AIR to run over the tool and into the dish.
7. Transfer the tissue and MEM-AIR scissor wash media by pouring into a new 50 mL conical tube.
8. Wash the dish 2 more times with 10 mL of MEM-AIR to remove all possible cells and again transfer to the conical tube.
  - a. Total volume of conical = 30mL.
9. Repeat steps 8-12 with second batch of tissue (if preparing a full prep).
10. Spin @ 2,000 RPM for 5 minutes.
11. Aspirate the supernatant (By bringing liquid to you...).
12. Re-suspend tissue with 10 mL of the filtered enzyme solution and swirl by hand to keep tissue in suspension.

13. Quickly pour into a sterile flask with stir bar.
14. Wash the conical tube with another 10 mL of enzyme solution. Keep it moving... pour into the flask.
15. Repeat with a third and final 10 mL of enzyme solution and again add to the flask. Total volume of flask = 30 mL
16. Repeat 15-18 for second half of prep if necessary.
17. Place the tissue / enzyme mixture flask in a circulating 37 °C water bath for 60 minutes...
  - a. Do not digest for more than or less than 60 minutes! This time is very important...
  - b. Also, be very careful with the temperature as well...around 36 °C is better than over 37 °C.
18. Prepare 100 mL of plating media:
  - a. 10 mL of dFBS
  - b. 5 mL of NuSerum
  - c. 2 mL of Kana-Gln
  - d. 1 mL of Gentamycin
  - e. 1 mL of Fungizone
  - f. 80 mL of MEM-D-Val
  - g. 10 µL of Ara-C
  - h. 25 µL of NGF
    - i. Filter with .22 micron filter
    - ii. Keep warm in 37°C water bath until needed
19. After the 60 minute incubation, remove flask from water bath.
20. Wet metal screen with a small amount of enzyme/cell solution. Filter the rest of the contents of the flask through the screen (this removes any bone / septum pieces).
21. Move tissue around on screen with rubber policemen to get all of the solution through and into a 50 mL conical tube.
22. Add 5 mL of dFBS to inactivate the enzymes.
23. Repeat 17-22 with second batch of tissue if necessary.
24. Spin at 1,000 RPMs for 5 minutes.
25. Remove supernatant via aspiration.
26. Reconstitute cell pellet in 5 mL of MEM-D-Val.
27. Filter through 70 micron cell strainer.
28. Repeat 25-27 with second batch of tissue if necessary.
29. Spin at 1,000 RPMs for 5 minutes.
30. Aspirate supernatant.
31. Reconstitute cell pellet (should be red on bottom and creamy white on top) in 10 mL of plating media. Triturate 10 times and add an additional 10 mL of plating media. Triturate again until all pellet is dissociated in the media.
32. Set up a final filter system using a 30 mL syringe and a Spectra (sterile) 10 micron mesh filter.
33. Repeat 30-33 with second batch of tissue if necessary.
34. Count and Plate:
  - a. To count:
    - i. Make a 10x dilution of the final filtered cell suspension (Step 32).
      1. 20 µL of cell solution into 180 µL of MEM-D-Val in a small eppendorf tube.
    - ii. Add 10-15 µL of this dilution to the hemacytometer. First half prep on one side, second half on the other (if necessary).
    - iii. Count and record numbers...

- iv. Take an average of the total cell count recorded and place into the following equation:

$$\frac{\text{Avg. cell count} * 20 \text{ mL (volume)}}{10 \text{ (dilution factor)}} = X$$
$$\frac{X}{2.0 \text{ million cells / mL (desired density)}} = Y$$

Y – 20 mL = Volume of plating media to be added to reach desired density.

**After Prep:**

1. Feed cells using feeding media (recipe below) once every day.
2. Add NGF and Ara-C (if desired) to the media
  - a. For 50mL of feeding media:
    - i. 10  $\mu$ L of 100 mM Ara-C
    - ii. 12.5  $\mu$ L of 2.5 ng/mL NGF
35. Remove one half of the media from the wells and replace with feeding media.
  - a. Never remove all of the media from the cells (a 50% change is fine).

**Medias:**

**MEM-D-Val**

9.28 g of powder media  
2.2 g of sodium bicarbonate  
0.292 g of L-glutamine  
Add double de-ionized water up to 1 L  
Stir and pH to 7.4  
Filter sterile and store at 4°

**MEM-AIR**

1 package of DMEM high glucose  
4.8 g of Hepes sodium Salt  
Add double de-ionized water up to 1 L  
Stir and pH to 7.4  
Filter sterile and store at 4°C

**15% Feeding Media:**

Add to 410 mL of MEM-D-Val:  
75 mL of dFBS  
10 mL of Kanamycin Sulfate  
5 mL of Gentamycin  
2.5 mL of Pen-strep  
Mix well  
Filter sterile and store at 4°C

**0.5% Feeding Media:**

Add to 480 mL of MEM-D-Val:  
2.5 mL of dFBS  
2.5 mL Pen-strep  
10 mL of Kanamycin sulfate  
5 mL of Gentamycin  
Mix well  
Filter sterile and store at 4°C

**Product Ordering Information:**

Ara-C, C6645, Sigma  
BSA, A7888, Sigma  
Collagenase D, 1088882, Roche  
FBS (Dialyzed), 26400-061, Life Technologies  
Dispase II, 165859, Roche  
DNase I, D4513, Sigma  
Fungizone, 152900187, Invitrogen  
Gentamycin, G3632, Sigma  
HEPES (Hemi) Salt, H7637, Sigma  
Hyaluronidase, H3884, Sigma  
Kanamycin, K4000, Sigma  
Laminin (Mouse), 354232, Becton Dickson  
MEM-AIR, 12100-046, Invitrogen  
MEM-D-Val, 99-909-PB (Special Order), Media Tech (Cellgro)  
NGF, 100700, Roche  
NuSerum, 355504, Collaborative BioMed  
10 micron filter, 148102, Spectra  
30 mL syringe, 309650, Becton Dickson  
70 micron cell strainer, 352350, Becton Dickson