**Post natal mouse neuron prep**

1. Set up an eppendorf for each pup with 50uL of HibernateA in each tube; place tubes on ice
2. Set up a 60mm petri dish for each pup with 7 mL dissection media in each; put on ice
3. Make the Papain solution:
	1. Weigh out papain
	2. Add HibA
	3. Incubate 37 deg C for 5 min
	4. Add DNAse
	5. Filter through syringe filter and place on ice

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| --- | --- | --- | --- | --- |
| # pups | Total solution (mL) | Papain (mg)  | Hib A (mL) | DNAse (mL)  |
| 1 | 1.5  | 3 | 1.2 | 0.15 |
| 2 | 2 | 4 | 1.6 | .2 |
| 3 | 2.5 | 5 | 2 | .25 |
| 4 | 3 | 6 | 2.4 | .3 |
| 5 | 3.5 | 7 | 2.8 | .35 |
| 6 | 4 | 8 | 3.2 | .4 |
| 7 | 4.5 | 9 | 3.6 | .45 |
| 8 | 5 | 10 | 4.0 | .5 |
| 9 | 5.5 | 11 | 4.4 | .55 |
| 10 | 6 | 12 | 4.8 | .6 |
| 11 | 6.5 | 13 | 5.2 | .65 |
| 12 | 7 | 14 | 5.6 | .7 |
| 13 | 7.5 | 15 | 6.0 | .75 |
| 14 | 8 | 16 | 6.4 | .8 |

1. Using a new Petri dish for each animal, dissect out hippocampi and place in eppendorf with Hibernate A on ice. Wash tools with 70% ethanol after each animal. When you are ready to dissect the last animal, place papain solution in water bath to warm up
2. Add 450 uL of the pre-warmed Papain solution to each tube; incubate at 37C on the nutator for 18 minutes
3. Let hippocampi settle to bottom of the tube; aspirate off Papain solution leaving about 50-100 ul liquid in bottom of the tube
4. Wash hippocampi 2x with 1mL each of Plating media
5. Add 1 mL Plating media to each tube; triturate hippocampi using a P200 with a 200 ul tip (use a new tip for each sample)
6. Let tissue settle to bottom of tube
7. Tirturate each sample again with a fire-polished glass pipette (use a new pipette for each sample)
8. For each sample, plate 4 wells of a 12-well plate with coverslips (pretreated with Poly-L-Lysine and washed with water; remove water and add 1mL plating media to each well before plating neurons)
9. Let neurons incubate 3-4 hours
10. Aspirate plating media and add 1mL Growth media (no araC)to each well
11. After 5 days, feed neurons with 1mL each Growth media + 10uM AraC (final concentration will be 5uM AraC)
12. Feed every 5-7 days by removing about 1mL condition media and adding mL fresh growth media + 5 uM AraC

**Coverslip preparation:**

Day 1:

1. Place coverslips (glass, 18mm) in jar with nitric acid and soak overnight

Day 2:

1. Pour off nitric acid.
2. Rinse with MilliQ water; pour off
3. Rinse again and let soak 1 hr on rocker
4. Repeat step 3.
5. Pour off water, add 100% ethanol; swirl, then pour off
6. Add more 100% ethanol. Store.

For treatment:

Day 1:

1. Take coverslips and allow to dry in hood on wattman paper for ~20 minutes
2. Transfer 1 coverslip per well of a 12-well plate using foceps
3. Thaw Poly-l-lysine, or dissolve new bottle of powder in 10 mL 0.1M Borate buffer to make a 10mg/mL stock
4. Dilute Stock in 0.1M Borate buffer to 1mg/mL in a volume equal to the number of coverslips you are treating.
5. Add 1 mL to each well, making sure coverslips are covered in Poly-L-Lysine solution; incubate over night at 37 deg C in TC incubator

Day 2:

1. Aspirate off Poly-L-Lysine
2. Add autoclaved MillQ water to each well (~1mL)
3. Let sit in hood or incubator for 1 hr (NO UV LIGHT)
4. Aspriate off water; repeat wash
5. After second wash, store coverslips in water in incubator until they are needed (aspirate off water before using)

**Recipes**

Borate Buffer

1.55g Boric Acid
2.375 g Borax
450 mL H2O
adjust to pH 8.5
QS to 500 mL with H2O

Dissection media:

200 mL H2O
20mL 1M HEPES, pH 7.55
20 mL 10x HANKS

Plating Media:

100 mL NBA
2 mL B27(2%)
1 mL 100x glutamax
10mL horse serum

Growth media:

200 mL NBA
4 mL B27
2 mL 100x glutamax

Feeding media:

200 mL NBA
4mL B27
2 mL 100x glutamaz
200 uL AraC