

## **Primary Low Density Hippocampal Culture**

### **Monday:**

Place coverslips into staining racks and place in a beaker.  
Rinse in ddH<sub>2</sub>O 2x10 min to remove dust and residual detergents.  
Wash coverslips in concentrated nitric acid for 18-36 hours.

### **Wednesday:**

Transfer staining racks to fresh beaker & rinse 6x20 min on shaker with Milli-Q ddH<sub>2</sub>O.  
Dry completely by vacuum aspiration or with a Kim-wipe  
*\*Dehydrate in 100 % EtOH twice - optional*  
*\*Flame coverslips, and keep them in a 120 mm plate - optional*  
Place coverslips in a beaker, cover with foil and bake at 225 °C for 6 hrs (Can also autoclave on dry cycle).

### **Friday (before the prep):**

Melt wax completely on hot plate at 90-110°C.  
In culture hood, put three wax spots (0.5 mm high) on each coverslip (5 coverslips/60mm dish).  
Irradiate under UV light for 30 minutes.  
Add 1 mg/ml of Poly-lysine (in borate buffer) to 60 mm culture plates.  
Keep O/N in 37°C incubator.

### **Monday:**

Wash the coverslips 4 x with ddH<sub>2</sub>O.  
Add 6 ml of astrocyte-conditioned medium (ACM) to each culture plate, and then keep in 37°C incubator.

### **Tuesday or Wednesday (Neuronal dissection/plating):**

Dissect E18 rat pups, remove hippocampi and place in 15 ml tube with 14 ml of dissection medium on ice.  
Let hippocampi settle to the bottom of the tube.  
Carefully aspirate the medium, but keep 2ml of medium on the hippocampi.  
Add 50ul of Papain (20 mg/ml in dissection medium) to digest the tissue (8 pups or 16 hippocampi).  
Incubate for 20 minutes at 37°C.  
Stop the digestion by washing the hippocampi 2x with 4 ml of complete (10% FBS) medium.  
Add 3ml of NB/B27, and dissociate the tissue by gently triturating the hippocampi through a fire polished Pasteur Pipette (3X3).  
Dilute the cell mixture to 10ml with NB/B27 and then filter with a 40 or 70 uM strainer.  
Spin the cells at 1800 rpm for 5 min. Re-suspend the cells in 10 ml of NB/B27.  
Count cells, and then plate at  $3.5 \times 10^5$ /dish.  
Incubate the cells for at least 3 hours (to overnight) at 37°C.  
Flip the coverslips into glial dishes containing 6 ml NB/B27 with FDU.  
At DIV 3, feed with NB/B27 (remove 2.5ml and add back 3 ml).  
Culture is then fed every Tuesday and Friday.

### **NM5 Medium:**

460 ml Neurobasal Media  
25 ml FBS  
10 ml Gluta-Max  
5 ml Pen/strep  
Keep the medium at 4°C

On the day of feeding: add 2% B27 (Gibco 17504044)

**Neuron Maintenance Medium:**

485 ml Neurobasal Media

10 ml Glutamax

5 ml Pen/strep

Add 2% B27 before using

**Product Ordering Information:**

Borate Buffer: 1 pk for 1 liter with ddH<sub>2</sub>O (Sigma T1194)

Poly-Lysine: Dissolve in ddH<sub>2</sub>O (Sigma P-2636)

Vitrogen (Pure-Col): Dilute with ddH<sub>2</sub>O to 1 mg/mL

Papain: make a stock with dissection buffer (Worthington 3120)

Coverslips:

18mm diameter/0.13–0.17 mm thickness Deckglaser German glass coverslips (*Carolina Biological*,

*Cat # 63-3033, \$11.10/Box of 100*)

15mm diameter/0.13–0.17 mm thickness (*Cat #63-3031, \$10.75/Box of 100*)

Coverslip staining racks (Thomas Scientific)

Paraplast (paraffin wax) (Sigma)