**LEE LAB RESEARCH LABORATORY**

**PRIMARY NEURON CULTURE PROTOCOL**

**Primary Hippocampal Neuron Culture Protocol**

1. Before dissection
	1. Dissection tools (scissors, forceps, spatulas, razor blades) should all be cleaned and autoclaved prior to use
	2. Prepare dishes or plates
		1. Minimum of 1 hr in 37C incubator
	3. Have solutions warmed, equilibrating, and prepared prior to starting dissection (plating media, digestion solution, digestion inhibition solution)
		1. Sterile filter digestion and inhibition solutions prior to use
	4. Flame polish autoclaved 9” Pasteur pipettes
2. Dissection
	1. In laminar flow hood: have aluminum foil for mice, Kimwipes or paper towels for dissection, tools, ice bucket and Brain Bits Hibernate A (BB HA)
	2. Begin dissection (steps may be done simultaneously on 6-8 pups or sequentially on each pup)
		1. Remove tools from alcohol
		2. Decapitate pup/s with scissors
		3. Use razor to make a mid-sagittal incision only penetrating the skin
		4. Use razor to make a small mid-sagittal incision in the skull, then press down hard hemisecting the brain and skull. Push apart.
		5. Dip blunt dissecting spatulas into the wash solution. Scoop out brain hemisphere, severing the olfactory bulb for ease
		6. Separate cortex from colliculi exposing the hippocampus.
		7. Identify the hippocampus by hallmark crescent shape, and medial, longitudinal blood vessel.
		8. Press down ventral of the hippocampus (in the crescent) with one spatula. Gently push onto this spatula with the other. The structure should cleanly slide free
		9. Place in chilled BB HA solution
	3. Keep on ice until ready to place hippocampi into warmed and sterile filtered digestion solution
3. Digestion
	1. Using 10 mL serological pipette, transfer hippocampi from BB HA to digestion solution
	2. Incubate in 37C water bath for 10-15 minutes, with intermediate mixing
	3. During this time:
		1. Ensure plates/dishes are ready
		2. Prepare trypan blue Eppendorf tube (150 uL TB + 50 uL cells) to count
4. Inhibition + Triturate
	1. Following digestion incubation, gently remove hippocampi with 10 mL serological pipette and place into 15 mL conical tube
	2. Wash hippocampi 3x with inhibition solution (3-4mL/wash)
	3. Then add final 4-5 mL inhibition solution and triturate hippocampi gently using fire polished pasteur pipette
	4. Once triturated, allow any undissociated tissue to sink to the bottom, gently transfer remaining suspension to fresh 15 mL tube.
	5. Pull 50 uL aliquot for counting, then centrifuge at 300 x g for 4 min @ 4C.
5. Count cells
	1. Make up the 200 uL (1:4 dilution of cells) trypan blue mixture, load 10 μL to hemacytometer, and count 4 quadrants
	2. Calculate desired concentration of cells/mL
6. Plate cells
	1. Dilute cells with appropriate amount of pre-equilibrated plating media to get desired cell concentration and then add to dishes/plates
	2. Place these in incubator and incubate for 3-4 hours, or overnight before switching to growth medium (Brain Bits NbActiv4)