



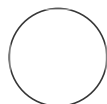
DEC 14, 2023

## PRIMARY NEURON CULTURE PROTOCOL

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**DOI:**  
[dx.doi.org/10.17504/protocols.io.14egn7jdmv5d/v1](https://dx.doi.org/10.17504/protocols.io.14egn7jdmv5d/v1)

**Protocol Citation:** Michael Lee 2023. PRIMARY NEURON CULTURE PROTOCOL. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.14egn7jdmv5d/v1>

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**Protocol status:** Working  
 We use this protocol and it's working

**Created:** Jun 04, 2022

**Last Modified:** Dec 14, 2023

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### ABSTRACT

This protocol details primary hippocampal neuron culture.

### ATTACHMENTS

[452-954.docx](#)

**Keywords:** primary  
Hippocampal neuron,  
dissection, Inhibition +  
Triturate, ASAPCRN

**Funders**  
**Acknowledgement:**  
ASAP

## MATERIALS

### Materials



- aluminum foil
- Kimwipes or paper towels
- ice bucket
- Eppendorf tube
- 15 mL conical tube
- alcohol
- Brain Bits Hibernate A
- trypan blue
- inhibition solution
- hemacytometer
- growth medium (Brain Bits NbActiv4)

### *Dissection tools*

- scissors
- forceps
- spatulas
- razor blades

## BEFORE START INSTRUCTIONS

### **Before dissection**

- Dissection tools (scissors, forceps, spatulas, razor blades) should all be cleaned and autoclaved prior to use.
- Prepare dishes or plates.
  - Minimum of  01:00:00 in  37 °C incubator.
- Have solutions warmed, equilibrating, and prepared prior to starting dissection (plating media, digestion solution, digestion inhibition solution).
  - Sterile filter digestion and inhibition solutions prior to use.
- Flame polish autoclaved 9" Pasteur pipettes.

## Primary Hippocampal Neuron Culture Protocol: 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.

#### Note

**Note:** Steps may be done simultaneously on 6-8 pups or sequentially on each pup.

- 2.1 Remove tools from alcohol.
- 2.2 Decapitate pup/s with scissors.
- 2.3 Use razor to make a mid-sagittal incision only penetrating the skin.
- 2.4 Use razor to make a small mid-sagittal incision in the skull, then press down hard hemisecting the brain and skull. Push apart.
- 2.5 Dip blunt dissecting spatulas into the wash solution. Scoop out brain hemisphere, severing the olfactory bulb for ease.
- 2.6 Separate cortex from colliculi exposing the hippocampus.
- 2.7 Identify the hippocampus by hallmark crescent shape, and medial, longitudinal blood vessel.
- 2.8 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.




2.9 Place in chilled BB HA solution.

3 Keep  On ice until ready to place hippocampi into warmed and sterile filtered digestion solution.

## Digestion

25m

4 Using 10 mL serological pipette, transfer hippocampi from BB HA to digestion solution.

5 Incubate in  37 °C water bath for  00:10:00 to  00:15:00, with intermediate mixing.

25m



6 During this time:

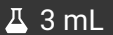
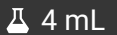
6.1 Ensure plates/dishes are ready.

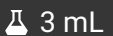
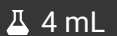
6.2 Prepare trypan blue Eppendorf tube ( 150 µL TB +  50 µL cells) to count.

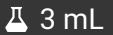
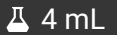
7 Following digestion incubation, gently remove hippocampi with 10 mL serological pipette and place into 15 mL conical tube.

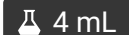
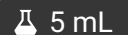
8 Wash hippocampi.



8.1 Wash hippocampi with inhibition solution  3 mL to  4 mL . (1/3)



8.2 Wash hippocampi with inhibition solution  3 mL to  4 mL . (2/3)

8.3 Wash hippocampi with inhibition solution  3 mL to  4 mL . (3/3)

9 Then add final  4 mL to  5 mL inhibition solution and triturate hippocampi gently using fire polished pasteur pipette.





10 Once triturated, allow any undissociated tissue to sink to the bottom, gently transfer remaining suspension to fresh 15 mL tube.

11 Pull  50  $\mu$ L aliquot for counting, then centrifuge at  300 x g, 4°C, 00:04:00 .

4m







## Count cells

- 12 Make up the  200  $\mu\text{L}$  (1:4 dilution of cells) trypan blue mixture, load  10  $\mu\text{L}$  to hemacytometer, and count 4 quadrants.
- 13 Calculate desired concentration of cells/mL.

## Plate cells

11h

- 14 Dilute cells with appropriate amount of pre-equilibrated plating media to get desired cell concentration and then add to dishes/plates.
- 15 Place these in incubator and incubate for  03:00:00 to  04:00:00, or  Overnight before  11h switching to growth medium (Brain Bits NbActiv4).

