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

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PRIMARY NEURON CULTURE PROTOCOL

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ABSTRACT

This protocol details primary hippocampal neuron culture.

ATTACHMENTS

452-954.docx

PROTOCOL integer ID:
63875

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

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

Inhibition + Triturate

4m

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 µL aliquot for counting, then centrifuge at 300 x g, 4°C, 00:04:00. 4m

Count cells

12 Make up the 200 µL (1:4 dilution of cells) trypan blue mixture, load 10 µL 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).