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Defined Media Primary Mouse Hippocampal Neuron Culture

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1 Works for me dx.doi.org/10.17504/protocols.io.bb47iqzn

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ABSTRACT

Culture of primary mouse hippocampal neurons. This protocol is for a defined media culture, meaning the use of a feeder layer is not required.

ATTACHMENTS

Defined Media Primary Neuronal Culture - Diaz Lab 2019.docx

Defined Media Primary Neuronal Culture - Diaz Lab 2019.pdf

1 Wash coverslips 3x with distilled water and place wax dots on coverslips

2 a.Transfer coverslips to 6-well plates (at least four; 24 coverslips total) in biosafety cabinet.

3 b.For sterility, turn on bunsen burner and place near hot plate.Sterilize bench area with 70% ethanol.

4 c.Begin boiling water in small beaker on hot plate at your bench.

5 d.Melt wax pellets by placing pellets into a smaller beaker.

6 e.Place this beaker into the water on hot plate as it begins to boil.

7 f.Bring coverslips to bench.Using 200 uL pipet, carefully add wax dots to the corners of each coverslip.

8 Transfer plates to bio-safety cabinet to dry

9 Genotype mice.

10 a.Use a sharpie to number each pup on the belly.

11 b.Snip a small piece of the tail and transfer to individually numbered tubes.

12 c.Digest tail snips to extract DNA and perform genotyping by following the Diaz Lab genotyping protocol.

13 d.Separate wildtype animals from knockout animals and proceed to dissection.

14 Perform hippocampal dissection and plating (see following protocol for details).

Day 6: (4DIV)

15 Add AraC (10 mM stock; 5 uM final concentration) per well directly to culture medium (1:2000 dilution from stock in -20C; i.e. 2.5 uL AraC to 5 mL NBC).

Day 7: (5DIV), **Day 12**: (10DIV), **Day 17**: (15DIV), **Day 21**: (20DIV)

16 Remove

17 µL of media, and replace with 1 mL fresh NBC.

Hippocampal Dissection and Plating

1. Before dissection, sterilize tools at 250°C for 15 minutes.

2. Decapitate P0-P2 pups, and remove brains from skull, placing in dissection buffer on ice.

3. After removing all brains, remove the hippocampi from each brain being careful to remove the meninges, and transfer to a 15mL conical tube on ice.

4. Transfer hippocampi to new 15 mL conical tube containing 5 mL of HBSS and 100 µL of 2.5% Trypsin (Gibco).
*** Add

18 µL papain (Wellingsworth) to 5 mL HBSS. Warm in 37°C water bath to aid in dissolving. Filter sterilize before use.

8. Incubate hippocampi in 37°C water bath for 12 or 15 minutes, inverting gently every few minutes.

9. After incubation, add 5mL of NPM.

10. Centrifuge for 5 min at 1000 rpm and carefully remove the supernatant.

11. Resuspend pellet in 3 mL of NPM and dissociate cells by pipetting up and down with a 1mL pipette 3x or 5x, followed by trituration with a fire polished long stem pipette until most chunks of tissue have been dissociated. For best results with mouse neurons do not triturate with glass pipette more than 12x. If there are tissue chunks remaining, let them settle to the bottom of the conical tube and then remove them.

12. Add NPM to a final concentration of 5-10 mLs.

13. Use a hemocytometer to determine cell density (45 uL of cell suspension plus 5 uL of trypan blue). Trypan blue will stain dead cells blue. Do not include dead cells in your count. For mouse neurons, 80% of your cells should be viable at this stage.

14. Dilute cells to 25,000/ mL (75,000 cells/ mL) with NPM in a separate container (make sure to account for pipetting error). Add 2 mLs per well, over the poly-L-lysine coated coverslips. This will give a final cell density of 50,000 (150,000) cells per well.

15. Place dishes in incubator for 5-6 hrs.

16. Replace media with NBC.

Media Formulations:

Dissection Buffer

19 ·500 mL HBSS (remove 10 mL)

20 ·5 mL Hepes

21 ·5 mL Sodium Pyruvate

Neuronal Plating Media (NPM)

22 ·500 mL MEM (remove 68 mL)

23 ·10% Donor horse serum (50mL)

24 ·0.45% glucose (7.5 mL of 30% stock in DI water)

25 ·5 mL Sodium Pyruvate

26 ·5 mL Penicillin/ Streptomycin

27 0pt;line-height:115%;font-family:"Times New Roman",serif'>Neurobasal complete (NBC)

500 mL Neurobasal (remove 27 mL)
5 mL Hepes
5 mL Sodium Pyruvate
5 mL Penicillin/ Streptomycin
1.5 mL L-glutamine
****Filter sterilize****
Add

28 mL B-27 after filter sterilizing

Additional Solutions:
Poly-L-Lysine Solution (PLL):
PLL stocks are at 200 mg/mL located in the -20C fridge.
For 25 ug/mL:

29 ·Dilute stock at 1:8,000 in 0.1 M Borate Buffer; i.e. add 6.25 uL PLL to 50 mL 0.1M Borate Buffer
For 1 mg/mL:

30 ·Dilute stock at 1:200 in 0.1 M Borate Buffer; i.e. add 50 uL PLL to 10 mL 0.1 M Borate Buffer
0.1 M Borate Buffer

31 ·900 mL milli-Q water

32 ·17.2 g sodium tetra-borate

33 ·3.1 g boric acid

34 ·Adjust pH to 8.5

35 ·Bring up to 1 L with milli-Q water

36 ·Filter Sterilize

1M Nitric Acid:

37 ·Add

38 mL 70% w/w 15.8 N nitric acid to 250 mL H_20.

39 ·Bring up to 1000 mL.