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Hippocampal Neuronal Culture

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ABSTRACT

This protocol describes the procedure for hippocampal neuronal cultures from new - born mouse pups.

ATTACHMENTS

fy7cbpdyf.pdf

MATERIALS

Solutions to prepare

1. Borate buffer (filter sterilize and keep at 4 °C)

[M] 50 millimolar (mM) boric acid, [M] 10 millimolar (mM) Sodium tetraborate decahydrate in DW, adjust to pH 8.5

2. Poly-D-Lysine (PDL) solution (filter sterilize)

- Dilute PDL to [M] 0.1 mg/mL in Borate Buffer.

3. Complete HBSS (filter sterilize)

[M] 10 millimolar (mM) HEPES pH 7.33, [M] 1 millimolar (mM) sodium pyruvate, 0.5% glucose, [M] 50 U/ml penicillin, [M] 50 mg/mL streptomycin in HBSS, adjust to pH 7.33.

4. Starting Medium (filter sterilize)

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[M] 1 millimolar (mM) sodium pyruvate, 0.5% glucose in MEM. This solution can be stored at 4 °C.

5. Plating Medium (100 mL, filter sterilize)

B-27 2 mL, glutamax 0.6 mL, FBS 10 mL and starting medium 87.4 mL

6. Trituration solution (20 mL)

Glutamax 0.1 mL, FBS 2 mL, 10% BSA/HBSS 2 mL in complete HBSS, adjust to pH 7.33 (Just before use, add DNase and filter it)

7. Complete Neurobasal Medium (100 mL, filter sterilize)

B-27 2 mL, glutamax 0.25 mL and Neurobasal 97.75 mL

8. Papain solution (Prepare fresh and Incubate at 37 °C for 00:30:00 to dissolve papain. Just before use, add DNase and filter it)

200 µL Papain (final concentration: [M] 20 U/ml) in 10 mL complete HBSS

Protocol

6h 30m

1 Coat MatTek dishes with 1.5 mL of PDL solution for 03:00:00 at 37 °C. 3h

2 Wash dishes twice with culture grade water and incubate the dishes at 37 °C for 00:30:00. 30m

3 Wash the dishes one more time and let dry. You can keep the dishes at 4 °C for few weeks (In this case, seal the dishes with paraffin film).

4 Just before starting neuronal culture, prepare papain solution and leave at 37 °C water-bath.

5 Dissect hippocampi from P0 (postnatal day 0) new-born mouse brains using a stereo microscope. Collect tissue in ice cold complete HBSS.

Note

From this step on everything is done under a sterile hood.

6 Aspirate medium and wash 2-3 times with 10 mL fresh ice cold complete HBSS.

7 Add DNAse (final concentration: [M] 20 µg/ml) to papain solution and filter sterilize.

Note

Note: Make sure it is completely dissolved. Incubate tissue prep with papain solution for 00:20:00 at 37 °C on a rocking platform.

8 Add DNAse (final concentration: [M] 20 µg/ml) to trituration solution and filter sterilize.

9 Aspirate the enzyme solution and wash twice with trituration solution

10 Resuspend samples in 1 mL trituration solution. Gently dissociate neurons with a P1000 filter tip by pipetting up and down for 10-12 times. Avoid generating any bubbles.

11 Count the cells.

> **Note**
>
> Form this step, all solutions don't include antibiotics. Must be careful to avoid contamination.

12 Seed the cells on the PDL coated MatTek dishes in pre-warmed Plating Media (2 mL).

(0.4 million cells / one MatTek dish).

13 After 03:00:00 incubation at 37 °C and 5% CO2, change the plating medium to pre-warmed Complete Neurobasal Media. 3h

14 Remove 500 µL of media and add 750 µL of fresh complete neuronal media at DIV (days in vitro) 4, 7 and 14. Incubate the media at 37 °C before adding it to neurons.