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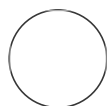
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Hippocampal Neuronal Culture

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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)

[1M] 50 millimolar (mM) boric acid, [1M] 10 millimolar (mM) Sodium tetraborate decahydrate in DW, adjust to pH 8.5

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

- Dilute PDL to [1M] 0.1 mg/mL in Borate Buffer.

3. Complete HBSS (filter sterilize)

[1M] 10 millimolar (mM) HEPES pH 7.33, [1M] 1 millimolar (mM) sodium pyruvate, 0.5% glucose, [1M] 50 U/ml penicillin, [1M] 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: $20\text{ }\mu\text{g/ml}$) 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: $20\text{ }\mu\text{g/ml}$) 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




From 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.

