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Protocol for hippocampal neuronal cultures

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ABSTRACT

This protocol details the procedure for preparation of neuronal cultures from mice hippocampi as it was performed in https://doi.org/10.1083/jcb.202010004 but can also be used to prepare cultures of cortical neurons.

ATTACHMENTS

dn3ubgtzx.pdf

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PROTOCOL CITATION

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MATERIALS TEXT

Solutions to prepare

Poly-D-Lysine (PDL)

- Dilute PDL to [M]0.1 mg/ml in Borate Buffer [M]0.1 Molarity (M) , pH8.5 and filter sterilize

Plating Medium: (filter sterilize and keep stable for 3 weeks at 4 °C)

A	B
Neurobasal (Gibco)	183 ml
FBS (Gibco)	10 ml (to final concentration of 5%)
Glutamax (Gibco)	2 ml
B27 (Gibco)	4 ml
Penicilin (Gibco)	0.5 ml (to final concentration of 50U/ml)
Streptomycin (Gibco)	0.5 ml (to final concentration of 50mg/ml)

Neuronal HBSS (nHBSS; filter sterilize and keep stable for 3 weeks at 4 °C)

A	B
HBSS (Gibco)	490 ml
1M HEPES (Gibco)	6 ml
100mM Pyruvic Acid (Gibco)	6 ml
H2O	100 ml
Penicilin (Gibco)	1.5 ml (to final concentration of 50 U/ml)
Streptomycin (Gibco)	1.5 ml (to final concentration of 50 mg/ml)

Neuronal Medium (filter sterilize and keep stable for 3 weeks at 4 °C)

A	B
Neurobasal (Gibco)	193 ml
Glutamax (Gibco)	2 ml
B27 (Gibco)	4 ml
Penicilin (Gibco)	1.5 ml (to final concentration of 50 U/ml)
Streptomycin (Gibco)	1.5 ml (to final concentration of 50 mg/ml)

Papain solution: (Prepare fresh and adjust pH if needed with [M]0.1 Molarity (M) NaOH, incubate at 37 °C for 00:30:00 to dissolve papain and filter)

A	B
Papain (Worthington)	200 ul (to final concentration of 20 U/ml)
nHBSS	10 ml
L-cysteine (Sigma)	2 mg (to 0.2 mg/ml)

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Protocol for hippocampal neuronal cultures 3h 30m

1 2h

Coat MatTek dishes with **1 mL** per dish of **0.1 mg/ml** Poly-D-Lysine (Sigma) for at least **01:30:00** to **Overnight** at **37 °C** .

2

Wash dishes twice with culture grade water and let dry.

3 30m

Prepare papain solution and leave at **37 °C** for **00:30:00** .

4

Dissect hippocampi from at least 3 P0 mouse brains using a stereo microscope. Collect tissue in ice cold Neuronal HBSS (nHBSS).

5 Transfer isolated hippocampi into a fresh cold nHBSS containing dish.

6 Cut tissue into ≈1mm^3 pieces and transfer into a **15 mL** Falcon tube with **10 mL** ice cold nHBSS and let sediment **On ice** .

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

7

Aspirate medium and wash 2-3 times with **10 mL** fresh ice cold nHBSS.

8 30m

Add DNAse to papain solution and filter sterilize. Incubate tissue prep with papain solution for **00:30:00** at **37 °C** on a rocking platform.

9

Aspirate the enzyme solution and wash twice with plating medium and then twice with nHBSS.

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10 Allow debris to settle for several minutes and collect supernatant.

Warning: Tissue will be softer after papain solution, handle with care to avoid dissociating the cells.

11 

Resuspend samples in **2 mL** cold nHBSS. Gently dissociate neurons with a P1000 filter tip by pipetting up and down for 10-12 times.

Warning: Avoid generating any bubbles.

12 Count the cells.

13 For imaging, seed 75,000 neuronal cells as a drop (usually around **100 µl**) in the PDLcoated coverslip of MatTek dishes in Plating Medium.

14 

3h 30m

After **03:00:00** to **Overnight** incubate at **37 °C** and 5% CO_2, change the plating medium to neuronal medium.

15 

Remove **500 µl** of media and add **1 mL** of fresh neuronal media every 3-4 days.

Suggestion: Incubate the media at **37 °C** and 5% CO2 before adding it to neurons.

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