Protocol for hippocampal neuronal cultures

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Solutions to prepare

Poly-D-Lysine (PDL)

- Dilute PDL to 0.1mg/ml in Borate Buffer 0.1M, pH 8.5 and filter sterilize

Plating Medium (filter sterilize and keep stable for 3 weeks at 4ºC)

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

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

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

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

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

Papain solution (Prepare fresh and adjust pH if needed with 0.1M NaOH, incubate at 37ºC for 30 minutes to dissolve papain and filter)

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

Protocol

1. Coat MatTek dishes with 1ml per dish of 0.1mg/ml Poly-D-Lysine (Sigma) for at least 1.5hrs to overnight at 37ºC.
2. Wash dishes twice with culture grade water and let dry.
3. Prepare papain solution and leave at 37ºC for 30 minutes.
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 15ml Falcon tube with 10ml 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 10ml fresh ice cold nHBSS.
8. Add DNAse to papain solution and filter sterilize. Incubate tissue prep with papain solution for 30 minutes at 37ºC on a rocking platform.
9. Aspirate the enzyme solution and wash twice with plating medium and then twice with nHBSS.
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 2ml 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 100ul) in the PDL-coated coverslip of MatTek dishes in Plating Medium.
14. After 3hr to overnight incubation at 37ºC and 5% CO_2, change the plating medium to neuronal medium.
15. Remove 500ul of media and add 1ml of fresh neuronal media every 3-4 days.

Suggestion: Incubate the media at 37ºC and 5% CO_2 before adding it to neurons.