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Primary Neuron Culture from Embryonic Rats

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Modified from Temkin et al., 2017. https://doi.org/10.1016/j.neuron.2017.03.020

Guidelines

This protocol may also be used with P0 or neonatal mouse pups.

Materials

NB Media:
-500 ml Neurobasal medium w/o glutamine (Gibco 21103-049)
-10 ml 50X B27 (ThermoFisher 17504044)
-5ml Glutamax (Gibco 35050-061)

Combine and sterile filter. Do not temperature cycle, pre-equilibrate aliquots as needed. Keeps for up to 2 months if stored at 4^{O}C.

Serum Media:
- 500 ml MEM w/ Earles salts w/o glutamine (Lonza 12125F)
- 25 ml FBS
- 1.912g Glucose
- 1ml Corning MITO+ serum extender (BD CB-50006)

Combine and sterile filter. Do not temperature cycle, pre-equilibrate aliquots as needed. Keeps for up to 2 months if stored at 4^{O}C.

Dissociation Media:
- 10 ml HBSS +$MgCl_2$/+$CaCl_2$ (Gibco 14025-092)
- 2mg L-cysteine (Sigma C7352), doesn't need to be precise, ~6-10 grains added
- 100ul 100mM CaCl2
- 100ul 50mM EDTA pH 8.0
- 20ul 0.5N NaOH
- 100ul papain (Worthington LS003126) vortex
- 100ul 10mg/ml Dnase I (Sigma D5025)

Pre equilibrate HBSS to 37^{O}C. Add in order of list, vortexing papain before addition. Sterile filter after papain dissolves and keep at 37^{O}C. Make no more than 2hrs before dissection.

Neutralization Media:
- 10ml Serum Media
- 25mg BSA culture grade (Sigma A4161-5G)
- 100ul 10mg/ml Dnase I (Sigma D5025)

Pre-equilibrate Serum Media to 37^{O}C. Sterile filter after BSA dissolves and keep at 37^{O}C. Make no more than 2hrs before dissection.

FUDR (200x stock):
- 100mg 5-FUDR (Sigma F0503-100mg)
- 250mg Uridine (Sigma u3003-5g)

- 50ml NBA Media

Dissolve, sterile filter, aliquot as 1ml, and freeze. Aliquots can be freeze/thawed.

Procedure

1 Prepare Dissociation and Neutralization media fresh and cool 15mL HBSS (+Mg/Ca) on ice.

2 Sacrifice time-pregnant rat (E18) by CO_2 asphyxiation followed by secondary method (thoracotomy or cervical dislocation).

Note: All procedures are performed in compliance with AAALAC guidelines and are approved by the Biogen Institutional Animal Care and Use Committee.

3 Wash lower abdomen with 70% EtOH and make incision to reveal embryos.

4 One at a time, remove embryos from amniotic sac and decapitate. Cut below brain on rostral or caudal side and gently peel off skull. Remove brain and transfer to ice-cold HBSS. Repeat until desired amount of tissue is collected (~$5x10^6$ cells/brain). Decapitate any unused embryos.

Optional: If there are significant delays between collection and dissection, you may use ice-cold Hibernate EB Complete Media (BrainBits HEB500) to extend viability. Delays between collection and dissection will impact cell viability.

5 Transfer brains to 10cm petri dish with ~5ml ice-cold HBSS.

6 Separate hemispheres, remove meninges and olfactory bulb, and dissect out desired tissue. Immediately transfer tissue to ice-cold HBSS and store on ice until all tissue is dissected.

7 Decant or aspirate HBSS and add 10mL warm Dissociation Media (up to 10 brains/10mL). Rock tissue at 37^{o}C from 30 minutes.

8 Allow tissue to settle and decant or aspirate Dissociation Media. Add 10mL warm Neutralization Media.

9 Remove all but 2mL of Neutralization Media, and gently triturate using P1000 pipette 10 times (avoid bubble formation). Let sit 2 minutes at RT.

10 Transfer supernatant (containing cell suspension) to new 15mL falcon tube, and add 2mL of Serum Media to the remaining settled tissue.

11 Repeat step 9, and then add the second cell suspension to the previously collected cells.

12 Spin down cell suspension at 30xg for 5 minutes, and then resuspend in 10mL of Serum Media.

13 Assess cell count and viability, and plate as needed on poly-D-lysine coated cultureware with NB Media.

14 Perform 50% media change using NB media every 4-7 days depending on seeding density.

15 Optional: Add FUDR at DIV4 to inhibit mitotic cell growth.