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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°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°C.

Dissociation Media:

- 10 ml HBSS +MgCl₂/+CaCl₂ (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°C. Add in order of list, vortexing papain before addition. Sterile filter after papain dissolves and keep at 37°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°C. Sterile filter after BSA dissolves and keep at 37°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 CO₂ 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 ($\sim 5 \times 10^6$ 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°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.