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Mouse primary cortical and hippocampal neuron preparation

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We use this protocol and it's working

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Abstract

This protocol describes preparation of cortical and hippocampal neurons from E15-E17 mouse embryos. These neurons may be maintained for at least 2 weeks. This in vitro model may be useful for studying neuronal physiology, viral transduction, or tool development.

IMPORTANT
This protocol is heavily adapted from https://app.jove.com/v/54981/reliable-identification-living-dopaminergic-neurons-midbrain-cultures. Review and watch the referenced protocol for greater insight into the procedures described.

Materials

Reagents

HBSS, no calcium, no magnesium, no phenol red **Thermo Fisher Scientific Catalog #**14175095

Papain from papaya latex **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P3125-250MG

HyClone donor equine serum, U.S. origin **Cytiva Catalog #**SH30074.03

Bovine Serum Albumin (BSA) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A7030-100G

DPBS with no calcium and magnesium **Thermo Fisher Scientific Catalog #**14190-144

Laminin Mouse Protein, Natural **Thermo Fisher Catalog #**23017015

Poly-L-Ornithine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P4957

POLY-D-LYSINE HYDROBROMIDE MOL WT 70000 - 5MG **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P6407-5MG

BrainPhys™ Neuronal Medium **STEMCELL Technologies Inc. Catalog #**05790

NeuroCult™ Neuronal Plating Medium **STEMCELL Technologies Inc. Catalog #**05713

NeuroCult™ SM1 Neuronal Supplement 10 mL **STEMCELL Technologies Inc. Catalog #**5711

UltraPure™ DNase/RNase-Free Distilled Water **Thermo Fisher Scientific Catalog #**10977023

Glutamax (100x) **Gibco - Thermo Fisher Scientific Catalog #**35050-061

L-Glutamic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G1251-100G

Consumables

0.2 µm sterile syringe filters (e.g.

Millex-GP Syringe Filter Unit, 0.22 µm **Merck Millipore (EMD Millipore) Catalog #**SLGP033RS)

Filtered pipette tips, including P1000 tips

Sterile 1.5 mL, 15 mL, and 50 mL tubes

Glass coverslips, if desired (e.g.

High performance coverslip 12mm dia 1.5h treated sterile super resolution **Neuvitro Corporation Catalog #**GG-12-1.5h-pre

)

Glass bottomed dishes, if desired (e.g. µ-Plate 24 Well Glass Bottom **Ibidi Catalog #**82427)

Sterile, non-TC treated 24-well plates

Sterile, 10 cm petri dishes for dissection

Sterile bottle or tube filters (e.g.

Steriflip-GP Sterile Centrifuge Tube Top Filter Unit **Merck Millipore (EMD Millipore) Catalog #**SCGP00525

)

Tools

4 pairs of fine-nosed forceps
2 pairs of dissecting scissors
No. 10 scalpel blade

Equipment
2 Chilled blocks or 2 ice buckets, with ice
Certified biosafety cabinet
Laminar flow hood
Bead sterilizer
Stereomicroscope and light source
Carbon dioxide euthanasia station

Troubleshooting

Reagent preparation

1 General note on reagent preparation:

> Note
>
> Prepare all reagents with sterile technique, and if possible, in a BSC.

2 Prepare poly-D-lysine solution [M] 0.1 mg/mL , by adding 50 mL of UltraPure water to 5 mg bottle of poly-D-lysine. Invert to mix and allow dissolution by leaving it at 4 °C Overnight .

Solution can be kept at 4 °C for at least a month.

3 Prepare laminin working solution [M] 0.02 mg/mL , by diluting laminin in appropriate volume of UltraPure water. Prepare enough for 200 µL per coverslip or well.

Prepare immediately before use.

> Note
>
> Thaw laminin slowly at 4 °C to prevent gelation. Upon receiving, we thaw then aliquot laminin to appropriate volumes, and store at -20 °C .

4 Prepare papain solution. Dilute papain to 15 units/mL in 1x HBSS. Pipette up and down 5 times to mix thoroughly.

Prepare on the day of dissection. For 4 cortical hemispheres (2 embryos), you will need 1 mL of papain solution.

5 Prepare stop solution. Dilute 1 mL donor equine serum to 10% in 9 mL 1x HBSS. Sterile filter with a 0.2 µm syringe filter tip. Store at 4 °C .

Prepare on the day of dissection.

6 Prepare 4% Bovine Serum Albumin (BSA) solution. Weigh out 200 mg of BSA and add 1x DPBS to a total final volume of 5 mL. Filter sterilize with 0.2 µm syringe filter tip. Store at 4 °C.

Prepare on the day of dissection.

7 Prepare complete maintenance media. Combine BrainPhys media and SM1 supplement. For 50 mL of complete media, combine 49 mL of BrainPhys media with 1 mL of SM1 supplement. Sterile filter.

Complete maintenance media can be stored in 4 °C for up to 1 month.

8 Prepare L-glutamic acid stock solution. Dissolve L-glutamic in UltraPure water to a concentration of [M] 2 mg/mL. Sterile filter, aliquot to appropriate volumes and store at -20 °C.

9 Prepare plating media. Combine NeuroCult plating media, SM1 supplement, Glutamax, and L-glutamic acid. For 10 mL of plating media, combine 9.8 mL of plating media, 200 µL of SM1 supplement, 25 µL of Glutamax, and 18.5 µL of L-glutamic acid. Sterile filter and store at 4 °C.

Prepare up to a few days before prep.

Coating coverslips or plate

3m

10 General note on coating:

> **Note**
>
> We found that coating glass wells or coverslips with poly-D-lysine, poly-L-ornithine and laminin yielded consistently healthy primary cultures. Other coating formulations may work better in your hands.
>
> Prepare coating and wash steps in a BSC. Begin coating 3 days before planned dissection.

11 (If using coverslips) In a sterile manner, transfer 1 coverslip to each well of a non-TC-treated 24 well plate.

12 Coat coverslips or well bottom with poly-D-lysine. Pipette 200 µL of poly-D-lysine solution ([M] 0.1 mg/mL). Incubate at 37 °C Overnight in a tissue culture incubator.

13 Aspirate poly-D-lysine solution. Perform 6 washes with UltraPure water, for 00:03:00 per wash.

Pipette 200 µL of poly-L-ornithine onto coverslip or well bottom. Incubate at 37 °C Overnight in a tissue culture incubator.

14 Aspirate poly-L-ornithine solution. Perform 3 washes with UltraPure water, for 00:03:00 per wash.

Pipette 200 µL of laminin onto coverslip or well bottom. Incubate at 37 °C Overnight in a tissue culture incubator.

15 Aspirate laminin solution. Perform 3 washes with DPBS, for 00:03:00 per wash. 3m

Pipette 300 µL of complete plating medium onto coverslip or well bottom. Incubate at 37 °C in a tissue culture incubator until use.

Prepare solutions and spaces

16 Prepare ice-cold 1x HBSS for dissection and papain solution. For 4 cortical hemispheres isolated from 2 embryos, you will need 20 mL of ice-cold 1x HBSS and 1 mL of papain solution. Keep both on ice until use.

Pre-warm plating media by transferring into a tissue culture incubator.

> Note
>
> Pre-warming media in an incubator allows the media to become appropriately buffered. Ensure that you thoroughly disinfect the media tube before placing into incubator.

17 Sterilize tools using bead sterilizer and disinfect the laminar flow hood.

18 Prepare 2 cooling blocks: one for holding the embryos still in utero and one for dissection of the cortex and further sectioning.

19 Prepare 4 petri dishes: one for embryos in uterus, one for dissecting tissue, one for the cortex and hippocampus post-dissection, and one for resting the sterile tools in.

20 Collect scalpel blade, P1000 pipette and sterile scissors to make wide-bore pipette tips.

Tissue collection

21 Euthanize a timed pregnant mouse on E15-E17 using CO2.

22 Sterilize the abdomen of euthanized mouse with 70% ethanol. Using surgical scissors, open the abdominal cavity and fold the abdominal wall over with forceps to expose the abdominal cavity.

23 Remove the uterus by cutting both ends of the uterine horn. Place the tissue into a Petri dish on a chiller. Transfer chiller and Petri dish to sterile laminar flow hood.

24 With forceps in each hand, open the embryo sac and remove the embryo. Decapitate the embryo using the scalpel blade. Position the head dorsal side up.

25 Using forceps in one hand, firmly grasp the snout to stabilize the tissue.

Using the forceps held in the other hand, gently remove the skin. Then using forceps, peel the skull off caudally along midline. Ensure that skull is adequately removed to allow for removal of the brain.

26 Once the brain is exposed, remove the brain from the skull.

Position forceps such that one tip is between the cortex and mesencephalon and the other is over the cerebellum. Gently grasp and lift out the entire brain, then place it in a Petri dish with ice-cold 1x HBSS, under a dissecting stereomicroscope and with adequate lighting.

Note

You may also remove the brain by gently overturning the skull and brain above the petri dish with ice-cold 1x HBSS, and teasing the brain out with forceps.

27 Repeat the procedure starting with step 21 for the remaining embryos.

28 Once all brains are collected, proceed with microdissection.

Under the dissecting microscope, orient the brain so that the dorsal side is accessible. Slip one set of forceps under either edge of the cortex, pinch and flip it away from the rest of the brain, leaving the hippocampus intact. Remove meninges and vasculature by gently grasping the meninges and pulling upward away from the brain. Pinch off olfactory bulb with forceps. Transfer the cortex with hippocampus to a clean petri dish with ice-cold 1x HBSS.

29 Repeat microdissection for remaining brains.

30 After all the brains have been microdissected, use forceps and a No. 10 scalpel to section each cortex into 8 pieces of approximately equal size.

Cell dissociation

31 Sterilely cut the tip off of a P1000 pipette tip, and use the tip to transfer the microdissected cortices and hippocampi to a 15 mL conical tube. Once the tissue has settled at the bottom, remove excess HBSS.

32 Transfer tube to sterile BSC.

33 Add 1 mL of papain solution to the tissue, and transfer to a 37 °C waterbath. Incubate for 00:15:00 , gently mixing the suspension by tapping halfway through.

34 Sterilely cut the tip off of a P1000 pipette tip.

Once the papain incubation is finished, use the wide bore tip to transfer the tissue from the papain solution into a new 15 mL conical tube with 1 mL of ice-cold stop solution.

Once the tissue has settled at the bottom, remove excess stop solution, then add 1 mL of fresh ice-cold stop solution. Repeat this rinse step 1 more time.

35 Create a single cell suspension by gently titurating cells with a P1000 pipette. Pipette cell suspension up and down 8 times.

Note

Avoid over tituration, as this may lead to poor cell viability.

36 Using P1000 pipette, transfer 800 µL of cell suspension to a new 15 mL conical tube. Avoid collecting any undissociated tissue segments. Underlay 200 µL of 4% BSA solution by slowly pipetting BSA into the bottom of the tube. Slowly and carefully withdraw the pipette tip to avoid disrupting the cell suspension.

Centrifuge the suspension at 280 x g, 18°C, 00:06:00 , then aspirate the supernatant with a P1000. Resuspend the cells in 1 mL of plating medium.

37 Count cells using a hemocytometer and further dilute to desired concentration with plating medium.

Note

We prefer to resuspend to the final concentration that will be in the well. For example, if you want to plate 60,000 cells in 300 µL , resuspend cells to a concentration of 200 cells/µL .

38 Plate cells. Aspirate plating media from wells/coverslips, and add appropriate volume of diluted cells.

39 Maintain the culture. We leave cells in plating medium for 5 days, then perform a half media change with complete maintenance media (alternatively, add equal volume of complete maintenance media). After this point, do a half media change every 3-4 days.

Protocol references

Henley, B. M., Cohen, B. N., Kim, C. H., Gold, H. D., Srinivasan, R., McKinney, S., Deshpande, P., Lester, H. A. Reliable Identification of Living Dopaminergic Neurons in Midbrain Cultures Using RNA Sequencing and TH-promoter-driven eGFP Expression. *J. Vis. Exp.* (120), e54981, doi:10.3791/54981 (2017).