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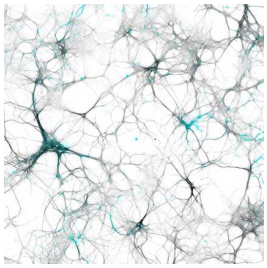
Preparation of primary hippocampal neurons



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ASAP Collaborative Rese… | West lab protocols

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We use this protocol and it's working

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Disclaimer

The **protocols.io** team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Abstract

This protocol details preparation of the primary hippocampal neuron culture. The protocol involves extraction ofdissecting hippocampi from rodent embryos P1 from nTg or transgenic mice, enzymatic digestion to dissociate cells, and seeding onto poly-D-lysine-coated dishes.

Attachments

DOCX

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23KB

Materials

Prepare solutions for plating

Borate buffer.

- [M] 50 millimolar (mM) , 3.09 g/L , pH 8.5
- Sterilize with 0.2 um filter.

Poly D lysine (PDL) hydrobromide (gibco, 0.1mg/ml, REF: A3890401. Life Technologies Corporation 3175 Staley Rd., Grand Island, NY 14072, USA):

	A	B
	40x stock	2 mg/mL
	Borate buffer pH 8.5	100 mg/50 mL

1. For 1x, dilute 40x with borate buffer.
2. Sterilize with 0.2 um filter.
3. Store in -20 °C .

Prepare solutions for dissection

> **Note**
>
> The same filter unit can be applied for each step of dissection

HBSS (Hanks' Balanced Salt Solution 1X) HBSS **Gibco - Thermo Fischer Catalog #**14025-092

	A	B
	HBSS	490 mL
	HEPES pH 7.4	6 mL
	pen/strep	3 mL
	100 mM pyruvic acid	6 mL
	distilled H2O	100 mL
	Total	600 mL

Filter (VWR Vacuum Filtration 250 ML 0.45 µm PES FILTER UNIT, Made in China Manufactured for VWR International, LLC 100 Matsonford Rd, Radnor, PA 19087)

Enzyme solution

	A	B
	HBSS	10 mL
	Papain suspension	200 µL
	L-cysteine	2 mg
	0.5 mM EDTA	22 µL

1. Rotate for 00:30:00 .
2. Filter (Millex-GP. Syringe-driven Filter Unit. 33mm, Pes Membrane, 0.22 µm, Sterilized. Merck Millipore Ltd. Tullagreen, Carrigtwohill, Co. Cork, IRELAND. Rev. 07/20).

Plating media (containing neurobasal media, supplemented with 5% FBS, 1x B27 supplement (ThermoFisher,17504044), [M] 0.5 millimolar (mM) L-glutamine, and 100 unit per mL of Penicillin-Streptomycin)

	A	B
	Neurobasal	183 mL
	FBS	10 mL
	GlutaMAX	2 mL
	B27	4 mL
	Penicillin-Streptomycin	1 mL

1. Filter (VWR Vacuum Filtration 250ML 0.45 µm PES FILTER UNIT, Made in China Manufactured for VWR International, LLC 100 Matsonford Rd. Radnor, PA 19087) and keep in 4 °C .
2. Warm Overnight /2 hours before in incubator in T75 flask (Thermo Fisher Scientific, Nunc^{TM} EasYFlask^{TM} 25 cm^2 Nunclon^{TM} Delta Surface.Nunc A/S. Kamstrupvej 90. P.O. Box 280 DK-4000 Roskilde. Denmark) to calibrate pH.

Neuronal/culture media (containing neurobasal media supplemented with B27 and [M] 0.5 millimolar (mM) L-glutamine)

	A	B
	Neurobasal	193 mL
	GlutaMAX	2 mL
	B27	4 mL

1. Filter (VWR Vacuum Filtration 250ML 0.45 µm PES FILTER UNIT, Made in China Manufactured for VWR International, LLC 100 Matsonford Rd. Radnor, PA 19087).
2. Warm Overnight /2 hours before in incubator in T75 flask to calibrate pH.

Materials

Poly-D-Lysine **Thermo Fisher Scientific Catalog #**A3890401

HBSS **Gibco - Thermo Fischer Catalog #**14025-092

Papain **Worthington Biochemical Corporation Catalog #**LS003126

B-27 Supplement **Gibco - Thermo Fischer Catalog #**17504044

Neurobasal™ Medium, minus phenol red **Thermo Fisher Catalog #**12348017

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

Protocol materials

Water sterile-filtered **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**RNBK1827 Step 1

Neurobasal™ Medium, minus phenol red **Thermo Fisher Catalog #**12348017 Materials

Glutamax (100x) **Gibco - Thermo Fischer Catalog #**35050-061 Materials

Poly-D-Lysine **Thermo Fisher Scientific Catalog #**A3890401 Materials

HBSS **Gibco - Thermo Fischer Catalog #**14025-092 In Materials, Materials

Papain **Worthington Biochemical Corporation Catalog #**LS003126 Materials

B-27 Supplement **Gibco - Thermo Fischer Catalog #**17504044 Materials

Nunc™ Cell-Culture Treated Multidishes 48 well **Thermo Scientific Catalog #**12565322 Step 1

Plate preparation

1 Wash 48 well plates

Nunc™ Cell-Culture Treated Multidishes 48 well **Thermo Scientific Catalog #**12565322

with distilled H_2O Water sterile-filtered **Sigma Aldrich Catalog #**RNBK1827

1.1 Wash 48 well plates with 0.5 mL distilled H_2O. (1/3)

1.2 Wash 48 well plates with 0.5 mL distilled H_2O. (2/3)

1.3 Wash 48 well plates with 0.5 mL distilled H_2O. (3/3)

2 Treat each well with 0.5 mL PDL at 37 °C for 01:00:00 . 1h

3 Wash with distilled H_2O.

3.1 Wash with 0.5 mL distilled H_2O. (1/3)

3.2 Wash with 0.5 mL distilled H_2O. (2/3)

3.3 Wash with 0.5 mL distilled H_2O. (3/3)

4 Place 0.5 mL plating media into each well at 37 °C .

Note

Note: This should be done right before dissection.

Dissection

5 Collect the hippocampi of 1-day postnatal CD1 mice pups.

6 Clean surgical instrument with 70% ethanol (Sharp fine scissors 14060-11 F.S.T, Sekmen Forceps, 11008-15 F.S.T).

7 Cut off head and using scissors, cut skin of the top of the head laterally.

8 Stabilizing head with tweezers, use another set of tweezers to pull skin to the side.

9 Cut through the bone.

10 Using a spoon, scoop brain from underneath and place into culture dish.

11 Separate the two hemispheres at the interhemispheric fissure from the brainstem.

12 Cut off the olfactory bulbs.

13 Carefully pull off the meninges but leave connected at the bottom.

14 Flip so that the bottom of the brain is facing up.

15 Completely pull off the meninges and associated tissues.

16 Hippocampus should be visible and make two cuts at either end.

17 Flip out hippocampus.

18 Trim hippocampus so that additional tissue is discarded.

19 Dissect hippocampus and using one pair of tweezers to push the tissue onto another, place hippocampus into the 15-mL tube with the 10 mL L-glutamin.

Cell culture

12h 45m

20 Wash with HBSS by pipetting to aspirate and NOT vacuuming.

20.1 Wash with 10 mL HBSS by pipetting to aspirate and NOT vacuuming. (1/2)

20.2 Wash with 10 mL HBSS by pipetting to aspirate and NOT vacuuming. (2/2)

21 Leave 1 mL HBSS in tube.

22 After filtering the enzyme solution with rotator, add all 10 mL of solution to the tube.

23 Incubate at 37 °C for around 00:45:00 and no longer than 1 hour.

45m

24 Remove media and leave 1 mL .

25 Pipette 10 mL plating media and 50 µL DNAse (DNAse stock is 50 µg/mL) into a 15-mL tube and invert 2-3 times.

26 Pipette 10 mL DNAse solution to the 1 mL digestion solution with hippocampi and invert 2-3 times.

27 Remove all media.

28 Wash with 10 mL plating media.

29 Wash with HBSS by pipetting to aspirate and NOT vacuuming.

29.1 Wash with 10 mL HBSS by pipetting to aspirate and NOT vacuuming. (1/2)

29.2 Wash with 10 mL HBSS by pipetting to aspirate and NOT vacuuming. (2/2)

30 Remove HBSS and leave 1 mL with HBSS with hippocampi.

31 Resuspend hippocampi with P1000 filter tip and pipet up and down 20 times until obvious chunks disappear.

32 Add 5 mL plating media.

33 Pass cells through strainer with 40 µm mesh size.

34 Count cells and plate 50,000 cells per well for a 48 well plate, and 25,000 cells per well for a 96 well plate.

34.1 Using cell counter.

1. Place glass slip on top of slide and pipette 10 µL cell solution under slip.
2. Count number of cells in one 4x4 grid (live cells appear round with dark ring and transparent inside)→# x 10^4 cells/mL.

34.2 Make sure not to add a small volume of cells to a large volume of plating media, aim for 250 µL of cell solution per well for a 48 well plate.

35 Leave plates in 37 °C incubator for 12:00:00 .

12h

36 Remove the plating media and swap with culture media containing neurobasal media supplemented with B27 and [M] 0.5 millimolar (mM) L-glutamine.

37 Culture the cells for an additional 7 days before use. At day-in-vitro (DIV) 10, add fibril strains to each well of neurons to a final estimated concentration of [M] 0.62 nanomolar (nM) (1 µg/mL).