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Postnatal primary hippocampal neuronal cultures

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We use this protocol and it's working

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Abstract

This protocol describes the preparation of postnatal primary hippocampal neuronal cultures.

Materials

- **Dissection solution (HBSS++) 500 mL**

	A	B
	491 mL	Hank's Balanced Salt Solution (HBSS) (UCSF Cell Culture Facility)
	5 mL	10 mM HEPES (UCSF Cell Culture Facility)
	4 mL	20 mM D-(+)-Glucose solution (G8769, Sigma)

- **Serum Neuronal Media (SNM) 25 mL**

	A	B
	22.8 mL	Minimal Essential Medium (MEM) (11090081, Gibco)
	213 µL	45% D-(+)-Glucose solution (G8769, Sigma)
	250 µL	Glutamax (3505006, Gibco)
		(Sterilize with a 0.22 µm syringe filter, and add the following reagents)
	1.25 mL	FBS (SH30070.02, Cytiva)
	500 µL	B27 (17504-044, Gibco)

- **Dissociation**
  Trypsin 0.25% (15090-046, Gibco)

- **Neurobasal Media (NBM) 25 mL**

	A	B
	24.175 mL	Neurolbasal Media (21103-049, Gibco)
	325 µL	Glutamax (35050061, Gibco)
		(Sterilize with a0.22 µm syringe filter, and add the following reagents)
	500 µL	B27 (17504-044, Gibco)

- **Other Supplies**

Warner Instruments 1.5 Glass coverslip 25 mm round, 54-0715
Cloning cylinders, 10×10 mm (CLS-1777-03, Chemglass Life Sciences)
Poly-L-lysine for coating (P2636, Sigma-Aldrich)

Plate and coverslip preparation and coating

1 Sterilize coverslips and clonal rings by submerging them in 70% (v/v) ethanol and briefly flaming them over a Bunsen burner.

2 Place sterile coverslips into each well of a 6-well plate.

3 Prepare clonal rings with grease applied to the bottom, and place each ring in the center of a coverslip.

4 Add poly-L-lysine (PLL) solution to cover the surface of each well (with coverslip and clonal ring in place). Incubate for 3 hours at 37°C in a 5% CO_2 incubator.

5 Remove PLL solution and wash the wells three times with sterile PBS.

Dissection and Dissociation

6 Dissect hippocampi from postnatal day 0–1 (P0-P1) mouse pups (appropriate genotypes) in HBSS++ solution.

7 Transfer tissue to 4.5 mL of HBSS++ containing 500 µL of 0.25% trypsin (15090-046, Gibco) in a 15 mL tube.

8 Incubate tissue in a 37°C water bath for 17 minutes for enzymatic digestion.

9 Wash tissue three times with SNM medium to remove trypsin.

Mechanical Dissociation

10 Add 1 mL of SNM to the tissue.

11 Using a glass Pasteur pipette of original diameter, triturate 3–4 times.

12 Flame the pipette to reduce its diameter by half. Triturate the tissue 5 times with the 1/2-diameter pipette.

13 Flame the pipette again to reduce its diameter to 1/4 of the original size. Triturate 5 times with the 1/4-diameter pipette.

14 Collect the 1 mL suspension of the resulting single cells and transfer to a 1.5 mL tube.

Cell counting and plating

15 Take 10 µL of the cell suspension and transfer to a 0.5 mL tube. Add Trypan Blue solution and count viable cells using a hemocytometer.

16 Plate dissociated neurons onto PLL-coated coverslips inside the 6-well plate at a density of 30,000–40,000 viable cells per well.

17 Incubate at 37°C, 5% CO_2.

18 On the day after plating, carefully replace 75% of the SNM with fresh, pre-warmed Neurobasal Medium (NBM) in each well using a 1 mL pipette.