

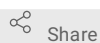


Aug 17, 2021

# Protocol for hippocampal neuronal cultures

Andrés Guillén-Samander<sup>1,2</sup>, Pietro De Camilli<sup>1,2</sup><sup>1</sup>Departments of Neuroscience and of Cell Biology, Howard Hughes Medical Institute, Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, Connecticut 06510, USA;<sup>2</sup>Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

1 Works for me



Share

[dx.doi.org/10.17504/protocols.io.bvgkn3uw](https://dx.doi.org/10.17504/protocols.io.bvgkn3uw)

Andrés Guillén-Samander

## ABSTRACT

This protocol details the procedure for preparation of neuronal cultures from mice hippocampi as it was performed in <https://doi.org/10.1083/jcb.202010004> but can also be used to prepare cultures of cortical neurons.

## ATTACHMENTS

[dn3ubgtzx.pdf](#)

## DOI

[dx.doi.org/10.17504/protocols.io.bvgkn3uw](https://dx.doi.org/10.17504/protocols.io.bvgkn3uw)

## PROTOCOL CITATION

Andrés Guillén-Samander, Pietro De Camilli 2021. Protocol for hippocampal neuronal cultures.

**protocols.io**<https://dx.doi.org/10.17504/protocols.io.bvgkn3uw>

## KEYWORDS

Hippocampus, Neuronal cultures, ASAPCRN

## LICENSE

This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

## CREATED

Jun 02, 2021

## LAST MODIFIED

Jul 02, 2021

## OWNERSHIP HISTORY

Jun 02, 2021 Urmilas

Jun 08, 2021 Andrés Guillén-Samander

## PROTOCOL INTEGER ID

50412

## MATERIALS TEXT

### Solutions to prepare

Poly-D-Lysine (PDL)

- Dilute PDL to **0.1 mg/ml** in Borate Buffer **0.1 Molarity (M)** , **pH 8.5** and filter sterilize

**Plating Medium:** (filter sterilize and keep stable for 3 weeks at **4 °C** )

A	B
Neurobasal (Gibco)	183 ml
FBS (Gibco)	10 ml (to final concentration of 5%)
Glutamax (Gibco)	2 ml
B27 (Gibco)	4 ml
Penicilin (Gibco)	0.5 ml (to final concentration of 50U/ml)
Streptomycin (Gibco)	0.5 ml (to final concentration of 50mg/ml)

**Neuronal HBSS** (nHBSS; filter sterilize and keep stable for 3 weeks at **4 °C** )

A	B
HBSS (Gibco)	490 ml
1M HEPES (Gibco)	6 ml
100mM Pyruvic Acid (Gibco)	6 ml
H2O	100 ml
Penicilin (Gibco)	1.5 ml (to final concentration of 50 U/ml)
Streptomycin (Gibco)	1.5 ml (to final concentration of 50 mg/ml)

**Neuronal Medium** (filter sterilize and keep stable for 3 weeks at **4 °C** )

A	B
Neurobasal (Gibco)	193 ml
Glutamax (Gibco)	2 ml
B27 (Gibco)	4 ml
Penicilin (Gibco)	1.5 ml (to final concentration of 50 U/ml)
Streptomycin (Gibco)	1.5 ml (to final concentration of 50 mg/ml)

**Papain solution:** (Prepare fresh and adjust pH if needed with **0.1 Molarity (M)** NaOH, incubate at **37 °C** for **00:30:00** to dissolve papain and filter)

A	B
Papain (Worthington)	200 ul (to final concentration of 20 U/ml)
nHBSS	10 ml
L-cysteine (Sigma)	2 mg (to 0.2 mg/ml)

1 

Coat MatTek dishes with **1 mL** per dish of **0.1 mg/ml** Poly-D-Lysine (Sigma) for at least **01:30:00** to **Overnight** at **37 °C**.

2 

Wash dishes twice with culture grade water and let dry.

3 Prepare papain solution and leave at **37 °C** for **00:30:00**.

30m

4 

Dissect hippocampi from at least 3 P0 mouse brains using a stereo microscope. Collect tissue in ice cold Neuronal HBSS (nHBSS).

5 Transfer isolated hippocampi into a fresh cold nHBSS containing dish.

6 Cut tissue into  $\approx 1\text{mm}^3$  pieces and transfer into a **15 mL** Falcon tube with **10 mL** ice cold nHBSS and let sediment **On ice**.

Note: From this step on everything is done under a sterile hood.

7 

Aspirate medium and wash 2-3 times with **10 mL** fresh ice cold nHBSS.

8 

30m

Add DNase to papain solution and filter sterilize. Incubate tissue prep with papain solution for **00:30:00** at **37 °C** on a rocking platform.

9 

Aspirate the enzyme solution and wash twice with plating medium and then twice with nHBSS.

10 Allow debris to settle for several minutes and collect supernatant.

**Warning:** Tissue will be softer after papain solution, handle with care to avoid dissociating the cells.

11 

Resuspend samples in **2 mL** cold nHBSS. Gently dissociate neurons with a P1000 filter tip by pipetting up and down for 10-12 times.

**Warning:** Avoid generating any bubbles.

12 Count the cells.

13 For imaging, seed 75,000 neuronal cells as a drop (usually around **100 µl**) in the PDLcoated coverslip of MatTek dishes in Plating Medium.

14  

3h 30m

After **03:00:00** to **Overnight** incubate at **37 °C** and 5% CO<sub>2</sub>, change the plating medium to neuronal medium.

15 

Remove **500 µl** of media and add **1 mL** of fresh neuronal media every 3-4 days.

**Suggestion:** Incubate the media at **37 °C** and 5% CO<sub>2</sub> before adding it to neurons.