Sep 09, 2022

Preparation of primary rat cortical neuron and astrocyte co-culture

mineechoi[1,2]

[1]Queen Square UCL Institute of Neurology; [2]The Francis Crick Institute

dx.doi.org/10.17504/protocols.io.n92ldzq1xv5b/v1

Minee-Liane Choi
UCL Institute of Neurology, The Francis Crick Institute

ABSTRACT

This protocol describes how to prepare primary rat cortical neuron and astrocyte co-culture.

DOI

dx.doi.org/10.17504/protocols.io.n92ldzq1xv5b/v1

PROTOCOL CITATION

mineechoi 2022. Preparation of primary rat cortical neuron and astrocyte co-culture . **protocols.io** https://protocols.io/view/preparation-of-primary-rat-cortical-neuron-and-ast-b9yjr7un

CREATED

May 25, 2022

LAST MODIFIED

Sep 09, 2022

PROTOCOL INTEGER ID

63211

MATERIALS TEXT

- Dissecting buffer: Hanks' Balanced Salt solution (HBSS) supplemented with [M]**10 millimolar (mM)** HEPES and [M]**20 % volume** Fetal bovine serum (FBS)
- Washing buffer: HBSS supplemented with 10mM HEPES
- Disgesting buffer: [M]**0.5 Mass / % volume** EDTA-trypsin supplemented with DNAse
- Neurobasal completed medium: Neurobasal A medium supplemented with B27, [M]**2 millimolar (mM)** Glutamax, Pen/Strep

1 1-3 days postpartum Sprague Dawley rats (University College London breeding colony) are used.
Experimental procedures are performed according to the United Kingdom Animal (Scientific Procedures) Act of 1986.

2 Rat cortices are placed in an ice-cold Dissecting buffer (described in Materials).

3 Wash five times with Washing buffer (described in Materials).

4 Tissues are digested with a Disgesting buffer (described in Materials) for **00:15:00** 15m

5 Digested tissues are neutralized with a dissecting buffer.

6 Washing twice with a Washing buffer,

7 Dissociate with a Washing buffer supplemented with DNAse.

8 Dissociated pellets are collected in Neurobasal completed medium.

Citation: mineechoi Preparation of primary rat cortical neuron and astrocyte co-culture
https://dx.doi.org/10.17504/protocols.io.n92ldzq1xv5b/v1

9 Approximately 600,000 cells are plated on **25 mm** Poly-D-Lysin (PDL) coated coverslips and 200,000 cells for 8-well ibidi chambers (PDL coated).

10 The cultures are maintained at **37 °C** (**5 % volume** CO2), and the media are changed every 4-5 days.

11 Cells can be used at 12-16 days.

Citation: mineechoi Preparation of primary rat cortical neuron and astrocyte co-culture
https://dx.doi.org/10.17504/protocols.io.n92ldzq1xv5b/v1