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PEI/Laminin Coating

In 1 collection

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dx.doi.org/10.17504/protocols.io.bu5iny4e

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ABSTRACT

This protocol is used to prepare plates for iPSC-derived neuron maturation. It is a part of the NGN2 cortical neuron differentiation protocol: coat plates in preparation for Day 7 dissociation and replating.

ATTACHMENTS

dh4fbiqa7.pdf

DOI

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PROTOCOL CITATION

Zoe 2021. PEI/Laminin Coating. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bu5iny4e

COLLECTIONS

HCNT-Cortical Neuron Transdifferentiation Overview

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PARENT PROTOCOLS

Part of collection
HCNT-Cortical Neuron Transdifferentiation Overview

MATERIALS TEXT

Materials

- **50 mL** Falcon tube

  Steriflip-GP Sterile Centrifuge Tube Top Filter
- Unit **Millipore Catalog #SCGP00525**
- **96 square-well plates (Brooks, #MGB096-1-2-LG-L)**
- Cell culture hood
- Multichannel pipet
- **Reagent Reservoir (Fisher Scientific, #21385104)**

Reagents

Pierce™ 20X Borate Buffer **Thermo**
- **Fisher Catalog #28341**
- Distilled water ()
- 5% PEI (poly-ethylenimine, stored at **4 °C**)
- Laminin **Sigma Catalog #L2020** , [M]**1 mg/ml**

  DPBS with Ca and Mg **Gibco - Thermo**
- **Fischer Catalog #14040-133**

  DPBS (Dulbeccos Phosphate Buffered Saline) 1x [-] calcium
- magnesium **Corning Catalog #21-031-CV**

  500 mL

PEI Coating

1 At least one day before plating, make 2x borate buffer with **4 mL** 20x borate buffer (Thermo, cat. no. 28341) in **36 mL** water in a **50 mL** Falcon tube.

2 Dilute **800 µl** 5% PEI (poly-ethylenimine; stored at **4 °C**) in **40 mL** 2x borate buffer to make 0.1% PEI.

3 Filter-sterilize with SteriFlip: attach filter and tube to **50 mL** tube with PEI solution; flip and attach to vacuum; when medium drains through, remove filter and original tube.

4 

Add **150 µl** to each of the inner 60 wells of a 96 square-well plate (Brooks, cat. no. MGB096-1-2-LG-L); **500 µl** for wells of 24-well plate (Corning, #3527).

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Note: Poly-ornithine-laminin pre-coated plates (6-well, 24-well) can be substituted for this plating protocol-only 96-well must be PEI/laminin coated.

5 On 96-well plates, leave a border of empty wells to avoid edge effects; fill these with PBS to maintain humidity.

6 

Label plate(s), wrap sides with parafilm and cover in plastic wrap; store at **4 °C** **Overnight** .

Laminin Coating

7 On the day of passaging, make laminin **5 µg/ml** in **4 °C** PBS (with calcium and magnesium ions), ex. **1 mL** PBS with **5 µl** laminin (Sigma, cat. no. L2020, **1 mg/ml**).

8 

Wash PEI-coated plates twice with distilled water (**300 µl** for 96-well plates).

9 

Wash once with PBS.

10 

Add **150 µl** laminin to wells of 96-well plate, and **0.5 mL** to wells of 12-well.

11 

Incubate plates at least 2 hours at **37 °C** before re-plating neurons.

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