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# Generation of human iPSC-derived tricultures

In 1 collection

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**We use this protocol and it's working**

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## Abstract

Generation of human iPSC-derived tricultures

## Materials

Final neuronal maturation medium: 1:1 DMEM-Ham's
F-12 and neurobasal medium, 0.5% N2, 1% B27 without vitamin A, 1% P/S, and 1%
GlutaMAX, supplemented with 200 µM AA, 1 µM purmorphamine, and 100 ng/mL FGF8
(PeproTech) + with 20 ng/mL BDNF (PeproTech) and 1
mM dibutyryl-cyclic AMP (dcAMP, Sigma–Aldrich).

## Generation of human iPSC-derived tricultures

1 Differentiate iPSCs into cortical neurons as per the established protocol ("Generation of human iPSC-derived cortical neurons, astrocytes, and microglia") until they reach 14 days in vitro (DIV).

2 At 14 DIV, dissociate neurons using Accutase for 5' at 37C

3 Plate dissociated neurons onto glass coverslips pre-coated with Matrigel 1:30 in DMEM 1X for 30' at 37C

4 Maintain plated neurons in neuronal maturation medium supplemented with 20 ng/mL BDNF and 1 mM dcAMP

5 After four days in culture, seed iPSC-derived astrocytes onto the neurons at a 3:1 ratio (neurons:astrocytes)

6 Maintain neuron-astrocyte cocultures in the final neuronal maturation medium

7 Three days after adding astrocytes, add iPSC-derived microglial precursors to the coculture at a 3:1:1 ratio (neurons:microglia:astrocytes)

8 Maintain the tricultures in the final neuronal maturation medium for an additional seven days in final neuronal maturation medium supplemented with 100 ng/mL IL-34, 100 ng/mL M-CSF, and 10 ng/mL GM-CSF