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Primary cortical astrocyte isolation and culture

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Shiyi Wang[1]

[1]Duke University

ASAP Collaborative Rese…

Shiyi Wang
Duke University

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Abstract

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1. P1 rat cortices from both sexes were micro-dissected, papain digested, triturated in low and high ovomucoid solutions, and resuspended in astrocyte growth media (AGM: DMEM (GIBCO 11960), 10% FBS, 10 µM, hydrocortisone, 100 U/ml Pen/Strep, 2 mM L-Glutamine, 5 µg/ml Insulin, 1 mM Na Pyruvate, 5 µg/ml N-Acetyl-L-cysteine).

2. Between 15-20 million cells were plated on 75 mm^2 flasks (non-ventilated cap) coated with poly-D-lysine and incubated at 37°C in 10% CO2.

3. On DIV 3, the removal of non-astrocyte cells was performed by forcefully shaking closed flasks by hand for 10-15 s until only an adherent monolayer of astrocytes remained.

4. AraC was added to the media from DIV 5 to DIV 7 to eliminate contaminating fibroblasts. On DIV 7, astrocytes were trypsinized (0.05% Trypsin-EDTA) and plated into 12-well or 6-well dishes.

5. On DIV 8, cultured rat astrocytes were transfected with shRNA and/or expression plasmids using Lipofectamine LTX with Plus Reagent (Thermo Scientific) per the manufacturer's protocol.

6. Briefly, 1 µg (12-well) or 2 µg (6-well) total DNA was diluted in Opti-MEM containing Plus Reagent, mixed with Opti-MEM containing LTX (1:2 DNA to LTX), and incubated for 30 minutes.

7. The transfection solution was added to astrocyte cultures and incubated at 37°C for 3 hours. On DIV 10, astrocytes were trypsinized, resuspended in NGM plus, plated (20,000 cells per well) onto DIV 10 neurons, and co-cultured for 48 hours.