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Rat astrocyte isolation and culture

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We use this protocol and it's working

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Abstract

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1 **Cortex Dissection and Digestion** - Micro-dissect P1 rat cortices from both sexes. - Digest cortices in papain solution.

2 **Trituration and Resuspension** - Triturate tissue in low and high ovomucoid solutions. - Resuspend cells 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

3 **Cell Plating and Incubation** - Plate 15-20 million cells on 75 mm² flasks (non-ventilated cap) coated with poly-D-lysine. - Incubate at 37°C in 10% CO2.

4 **Non-Astrocyte Cell Removal (DIV 3)** - Forcefully shake closed flasks by hand for 10-15 seconds to remove non-astrocyte cells. - Ensure only an adherent monolayer of astrocytes remains.

5 **AraC Treatment (DIV 5 to DIV 7)** - Add AraC to the media to eliminate contaminating fibroblasts.

6 **Astrocyte Passage and Plating (DIV 7)** - Trypsinize astrocytes with 0.05% Trypsin-EDTA. - Plate cells into 12-well or 6-well dishes.

7 **Transfection of Astrocytes (DIV 8)** - Transfect with shRNA and/or expression plasmids using Lipofectamine LTX with Plus Reagent (Thermo Scientific). - Dilute 1 µg (12-well) or 2 µg (6-well) total DNA in Opti-MEM with Plus Reagent. - Mix with Opti-MEM containing LTX (1:2 DNA to LTX ratio). - Incubate for 30 minutes at room temperature. - Add transfection solution to astrocyte cultures and incubate at 37°C for 3 hours.

8 **Co-Culture with Neurons (DIV 10)** - Trypsinize astrocytes on DIV 10. - Resuspend in NGM Plus. - Plate 20,000 cells per well onto DIV 10 neurons. - Co-culture for 48 hours.