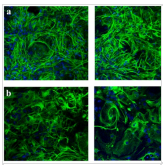

Isolated astrocyte culture - protocol 2

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Working

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ABSTRACT

An alternative protocol for isolated astrocyte cell culture preparation. Dissociation, preparation and plating of mice cortex neurons and glia cells on flasks. Isolatioin process of the culture from neurons, to obtain an isolated astrocyte culture. Plating isolated astrocytes on MEA.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

Sample collection

1 Dissect and place on ice cortices from pups (post natal day 0 or 1 mice).

Cell lysis

2 1. Chopp with scissors in a papain-based dissociation buffer (2.5 mM CaCl2, 0.83 mM EDTA, 137 U papain (Sigma-Aldrich)), 100 µl DNAse (Sigma-Aldrich), 3–5 crystals of L-Cysteine (Sigma-Aldrich), HBSS with 20 mM HEPES (pH 7.4);

2. Place on a rotating shaker for 15 min 00:15:00 at room temperature.

Cell preparation

3 1. After centrifuging, discard the supernatant , resuspend the pallet in modified essential medium (MEM) without L-glutamine with essential amino acids (Beit Haemek, 06-1025-01-1A), 5% heat-inactivated fetal calf serum (Biological Industries), heat-inactivated 5% horse serum (Beith Haemek, 04-004-1), 2 mM glutamine (Beit Haemek, 03-020-1c), 3 mg/ml glucose, 2% B-27 (Gibco, 17504-044), 0.5% Pen/Strep (100 U/ml penicillin, 100 µg/ml streptomycin; Beit Haemek, 03-031-1B);

2. Triturate seven times.

Cell culture

4 1. Plate the cells on poly-D-lysine (PDL, Sigma-Aldrich, P7405-5MG) coated T75 flasks;

2. Maintain cultures at 37 °C with 5% CO2. s Replace growth medium every 3-4 days to astrocyte enriched growth medium: MEM-EAGLE (without L-glutamine, with essential amino acids) (Beit Haemek), 3 mg/ml glucose, 10% heat-inactivated fetal calf serum (Biological Industries), 0.8% GlutaMAX (100X; Gibco), 0.5% Pen/Strep, 2 mM glutamine (Beit Haemek);

3. After 20 DIV detache using trypsin/EDTA;

4. Re-plate the cells on poly-D-lysine (PDL, Sigma-Aldrich, P7405-5MG) coated micro-electrode arrays (MEAs; 200/30iR-Ti-gr and 500/30iR-Ti-pr; Multichannel Systems) with a cell density of 2000-2500 cells/mm2 (~106 cells per dish).