

Neuron-astrocyte culture preparation

Yasmin Bar El¹

¹School of Physics and Astronomy, Tel-Aviv University, Tel-Aviv, Israel

Sep 21, 2018 dx.doi.org/10.17504/protocols.io.tqaemse

Working

GigaScience Journal



Yasmin Bar El

ABSTRACT

Primary cortical neuronal-astrocyte cell culture preparation. Dissociation, preparation and plating of mice cortex neurons and glia cells on MEA.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

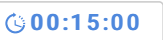
Sample collection

- 1 Dissect cortices from pups and place them on ice.

NOTE

Cortical cultures were prepared from post-natal day 0 or 1 mice.

Cell lysis

- 2 1. Chop 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 at 50 rpm for 15 min  00:15:00 at room temperature.

Cell preparation

- 3 1. After centrifuging (1000 rpm, 1 min), discard the supernatant, resuspend the pellet 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 (Beit 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 by slowly passing the tissue through a 10 mL serological pipette until the tissues are smaller than the opening of the 1 mL pipette tip.

Cell culture

- 4 1. 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);
2. Cultures were maintained at 37 °C with 5% CO₂. Partially replace growth medium every 3-4 days [MEM-EAGLE (without L-glutamine with essential amino acid), 5 mg/ml glucose, 5% heat-inactivated fetal calf serum, 0.8% GlutaMAX (100X; Gibco, 35050-038), 0.5% Pen/Strep, 2 mM glutamine, 2% B-27].



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited