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Immunocytochemistry with Primary Cultured Hippocampal Neurons

In 1 collection

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Mukesh Kumar[1], Timothy A. Ryan[1,2]

[1]Department of Biochemistry, Weill Cornell Medicine, New York, NY 10065;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, Maryland 20815, USA

Mukesh Kumar
Weill Cornell Medical College

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We use this protocol and it's working

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Abstract

This protocol describes a detailed method for performing immunocytochemistry (ICC) on hippocampal neurons cultured from post-natal day 0-1 pups.

Guidelines

The protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Materials

- Hippocampal neurons: DIV-14
- Poly-D-lysine hydrobromide (PDL) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P7886
- 4% paraformaldehyde (ThermoScientific, J19943.K2) + 10% sucrose (SigmaAldrich, S0389)
  Sucrose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S0389
- Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T8787
- Goat serum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G9023
- Primary antibodies:

DDHD2 Polyclonal antibody **Proteintech Catalog #**25203-1-AP ,

Synapsin1/2 antibody **Synaptic Systems Catalog #**106 004 ,

Monoclonal Anti-TOMM20 antibody produced in mouse **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**WH0009804M1

, Neuron-specific beta -III Tubulin Antibody **R&D Systems Catalog #**MAB1195 ,

NeuN (Abcam, ab104224, ab177487)

Anti-NeuN antibody [1B7] - Neuronal Marker **Abcam Catalog #**ab104224 ,

Anti-NeuN antibody [EPR12763] - Neuronal Marker **Abcam Catalog #**ab177487

GFAP (Abcam, ab7260 Anti-GFAP antibody **Abcam Catalog #**ab7260

GFAP Antibody **Novus Biologicals Catalog #**NB300-141),

GM130 (E9Z6S) Rabbit mAb #70767 **Cell Signaling Technology Catalog #**70767T

- Alexa Fluor-conjugated secondary antibodies (All from Invitrogen)
- BODIPY™ 493/503 **Life Technologies Catalog #**D3922
- MDH (Abgent, SM1000a)
- ProLong Diamond Antifade Mountant **Thermo Fisher Scientific Catalog #**P36970
- PBS, pH 7.4 **Thermo Fisher Catalog #**10010031

Equipment

- Dissection tools
- Humidified chamber (Custom made)
- Frosted microscope slides (Avantor, 16004-422)
- Confocal Microscope (Zeiss 880)

Neuronal Culture Preparation

1 Isolate hippocampal neurons from post-natal day 0-1 pups using standard dissection techniques.

2 Plate the neurons sparsely onto glass coverslips coated with poly-D-lysine.

3 Maintain neurons in culture media under standard conditions (37 °C , 5% CO_2) until they reach 14 days in vitro (DIV-14).

Fixation

25m

4 Prepare a fixative solution containing 4% PFA and 10% sucrose in PBS.

5 Fix the neurons by gently adding the fixative solution and incubating at Room temperature for 00:10:00 .

10m

6 Rinse the fixed neurons 3 times with PBS (5 minutes each wash).

6.1 Rinse the fixed neurons with PBS for 00:05:00 each wash (1/3).

5m

6.2 Rinse the fixed neurons with PBS for 00:05:00 each wash (2/3).

5m

6.3 Rinse the fixed neurons with PBS for 00:05:00 each wash (3/3).

5m

Permeabilization

20m

7 Permeabilize the fixed neurons by incubating in 0.25% Triton X-100 in PBS for 00:05:00 at Room temperature . 5m

8 Wash the permeabilized neurons 3 times with PBS (5 minutes each wash).

8.1 Wash the permeabilized neurons with PBS for 00:05:00 each wash (1/3). 5m

8.2 Wash the permeabilized neurons with PBS for 00:05:00 each wash (2/3). 5m

8.3 Wash the permeabilized neurons with PBS for 00:05:00 each wash (3/3). 5m

Blocking 1h 10m

9 Prepare a blocking solution containing 5% goat serum in PBS.

10 Incubate the neurons in the blocking solution for 01:00:00 at Room temperature . 1h

11 Gently wash the neurons once with PBS for 00:10:00 . 10m

Primary Antibody Incubation 10m

12 Dilute the primary antibodies in a solution containing 5% goat serum and 0.05% Triton X-100 in PBS, following the manufacturer's instructions.

13 Apply the primary antibody solution to the neurons and incubate Overnight at 4 °C in a humidified chamber. 10m

Washing

15m

14 Wash the neurons 3 times with PBS (5 minutes each wash).

14.1 Wash the neurons with PBS for 00:05:00 each wash (1/3). 5m

14.2 Wash the neurons with PBS for 00:05:00 each wash (2/3). 5m

14.3 Wash the neurons with PBS for 00:05:00 each wash (3/3). 5m

Secondary Antibody Incubation

1h

15 Dilute the Alexa Fluor-conjugated secondary antibodies (Alexa 488, Alexa 546, Alexa 647) to a 1:500 dilution in 0.05% Triton X-100 in PBS.

16 Apply the secondary antibody solution to the neurons and incubate for 01:00:00 at Room temperature in a humidified chamber. 1h

Washing and Lipid Droplet Staining

30m

17 Wash the neurons twice with PBS (5 minutes each wash).

17.1 Wash the neurons with PBS for 00:05:00 each wash (1/2). 5m

17.2 Wash the neurons with PBS for 00:05:00 each wash (2/2). 5m

18 For lipid droplet staining: Prepare a staining solution containing 1 µL BODIPY or [M] 100 micromolar (µM) MDH in PBS. Perform the third wash using this solution, 10m

incubating for 00:10:00 .

19 Gently wash the neurons with PBS for an additional 00:10:00 . 10m

Mounting

20 Carefully mount the glass coverslips onto glass slides using Prolong antifade mounting media containing BODIPY or MDH to prevent photobleaching.

21 Seal the coverslip with nail-polish to prevent drying.

Imaging

22 Use a confocal or fluorescence microscope to acquire images of the stained neurons.

Note

- Bovine Serum Albumin (BSA) can be used in place of Goat serum.
- Maintain gentle handling of neurons throughout the protocol to prevent damage of synapses.
- Protect fluorescently labeled samples from light to preserve signal intensity.
- Imaging conditions should be optimized to capture fluorescence signals without significant photobleaching.