Aug 02, 2023

Immunostaining of corticostriatal culture on fluid-walled dumbbells

DOI
dx.doi.org/10.17504/protocols.io.36wgqj8eyvk5/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.36wgqj8eyvk5/v1

Protocol Citation: Quyen Do, Federico Nebuloni, Richard Wade-Martins 2023. Immunostaining of corticostriatal culture on fluid-walled dumbbells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgqj8eyvk5/v1

Protocol status: Working
We use this protocol and it's working

Created: April 24, 2023

Last Modified: August 02, 2023

Protocol Integer ID: 80966

Keywords: Corticostriatal pathway, fluid-walled microfluidics, corticostriatal culture on fluid, derived corticostriatal culture, corticostriatal culture, walled dumbbell, walled dumbbells this protocol, immunocytochemistry

Funders Acknowledgements:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-020370

Abstract

This protocol describes the immunocytochemistry of the iPSC-derived corticostriatal culture on fluid-walled dumbbells.

Materials

Reagents:

- Citrate buffer solution, pH 6.0 (Sigma- Aldrich, SKU# C9999-100ML)
- Donkey Serum (Sigma- Aldrich, SKU# D9663-10ML)
- PBS with Azide (Insight Biotechnology, CA# sc-296028)
- Paraformaldehyde (PFA) (Sigma- Aldrich, CAT#P6148)
- Triton X-100 (Sigma- Aldrich, SKU# X100)

Troubleshooting

Before start

Adherent cells are prone to peeling, and hence, addition and removal of any liquid should be performed slowly with care.

At no point during the entire procedure prior to mounting should the sample be left to dry.

Fluorescent-dye conjugated Secondary Antibodies are light-sensitive and hence should always be protected from light.

All PBS washes should be at least 10 mins, and plates are left to incubate on the bench at room temperature.

Generation of the corticostriatal pathway coculture on fluid-walled dumbbells

1 Fabricate the fluid-walled dumbbells on 6 cm culture dishes as described in **step 1** of **Protocol: Fabrication of fluid-walled dumbbells and generation of the human corticostriatal pathway**

2 Allow cells to be cultured on glass coverslips as described in **Section: Establishment of Human Corticostriatal Pathway** in **Protocol: Fabrication of fluid-walled dumbbells and generation of the human corticostriatal pathway** until relevant experimental timepoints for immunocytochemistry.

PFA Fixation

3 At day 25 of the coculture, remove all cell media and FC40 by gentle pouring.

4 Wash the whole dish with PBS twice using P1000.

5 Fix cells by adding 2% PFA in PBS to each dish at room temperature for 20 mins.

6 Remove PFA completely using a pipette.

7 Wash thoroughly 3 times with PBS, at least 10 mins each.

8 Incubate the coverslips in PBS for at least 1 hour at room temperature or at 4°C overnight.

Heat-induced Antigen Retrieval

9 Perform antigen retrieval by adding 2 mL of 1x citrate buffer (pH 6.0) to each dish, and place the culture dish in a water bath at 80ºC for 5 mins.

Note

This step is time- and temperature-sensitive.

Fluid can quickly dried out during the incubation - a generous amount of citrate buffer should be added. Longer incubation and/or higher temperature can damage the cell sample.

10 Leave to incubate at room temperature for 10-20 mins.

11 Prepare blocking solution containing PBS with 10% Donkey Bovine Serum (NBS) and 0.1% Triton-X during the post-antigen retrieval incubation.

12 Remove the citrate buffer and add 1 mL of blocking solution.

13 Leave to incubate at room temperature for 10 mins.

14 Wash twice with PBS, at least 10 mins each.

Primary Antibody Incubation

15 During previous washes, prepare the Primary Antibody Solution containing PBS with 10% Donkey Bovine Serum (NBS), supplemented with appropriate primary antibodies in their respective working concentrations.

The following primary antibodies (working concentration 1:250) were used in Do, Q. et al. (2023) for immunostaining: anti-DARPP32, anti-DARPP32, anti-MAP2, anti-GFP.

16 Remove all the PBS from each well and add 80 mL of the Primary Antibody Solution.

17 Leave to incubate overnight at 4°C.

Secondary Antibody Incubation

18 Remove all the Primary Antibody Solution and add fresh PBS.

19 Wash thoroughly 3 times with PBS, at least 10 mins each.

20 During the last wash, prepare Secondary Antibody Solution containing PBS with 10% Donkey Bovine Serum (NBS), supplemented with appropriate secondary antibodies and DAPI (4',6-Diamidino-2-Phenylindole, Dilactate) in their respective working concentrations.

The following secondary antibodies (working concentration 1:1000) were used in Do, Q. et al. (2023) for immunostaining: Alexa-Fluor 555 Mouse, Alexa-Fluor 648 Rabbit, Alexa Fluor 488 Chicken, and DAPI.

Note

Keep pre-made solution wrapped in aluminium foil and/or keep away from direct light during incubation.

21 Remove all the PBS from each well and add 80 mL of the Secondary Antibody Solution.

22 Leave to incubate at room temperature for 1-2 hours.

Note

Keep cell plates wrapped in aluminium foil and/or keep away from direct light during incubation.

23 Once done, wash well with PBS.

24 Incubate the culture in generous amount of PBS Azide until imaging.