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We use this protocol and it's working

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Immunofluorescence staining

In 2 collections

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ABSTRACT

This protocol describes the immunofluorescence staining of cells.

ATTACHMENTS

404-872.docx

MATERIALS

Recipes and products:

	A	B
	8% Paraformaldehyde (PFA)	
	PFA	20 g
	1M NaOH	0.5 ml
	1x PBS	100 ml

Note

- Heat to ~ 60 °C to dilute. Then filter through folded filters into new cylinder.
- Adjust pH 7.4 (normally its ~ pH 7.38 without adding something)
- Fill up to 250 mL with 1x PBS.

Dako Mounting Medium

Fluorescence Mounting Medium **Agilent Technologies Catalog #S302380-2**
:

NGS (normal-goat serum)

Normal Goat Serum Blocking Solution **BIOZOL Catalog #VEC-S-1000**

Note

- Prepare aliquots out of stock and store in -20 °C.
- Filter before use to avoid contamination.

DAPI (DAPI (4',6-Diamidino-2-Phenylindole, Dilactate)

DAPI (46-Diamidino-2-Phenylindole Dilactate) **BioLegend Catalog #422801**:

- Dissolve the content in 2 mL deionized water (DAPI concentration [M] 10.9 millimolar (mM)).

Fixation of the cells: Strategy 1, i.e., HEK cells

25m

1 Remove medium.

2 Wash with 1× PBS.

3 Remove PBS and add 4% PFA to the cells.

Note

Note: For a coverslip in a 24 multi-well use at least 300 µL /well.

4 Incubate 00:10:00 at Room temperature .

10m

5 Collect PFA.

6 Wash 1× PBS.

6.1 Wash with 1× PBS for 00:05:00 at Room temperature . (1/3)

5m

6.2 Wash with 1× PBS for 00:05:00 at Room temperature. (2/3) 5m

6.3 Wash with 1× PBS for 00:05:00 at Room temperature. (3/3) 5m

Fixation of the cells: Strategy 2, i.e., neurons 25m

7 Add the same volume of 8% PFA as medium is in the well to the well.

8 Incubate 00:10:00 at Room temperature. 10m

9 Collect PFA in a falcon.

10 Wash in 1× PBS.

10.1 Wash with 1× PBS for 00:05:00 at Room temperature. (1/2) 5m

10.2 Wash with 1× PBS for 00:05:00 at Room temperature. (2/2) 5m

Note

Storage until ICC: Keep coverslips in 1× PBS, seal the plate with parafilm and store at 4 °C.

Blocking and permeabilization

25m

11 Remove 1X PBS.

12 Add 300 µL /well of blocking solution.

Note

Blocking solution: 10% NGS [normal goat serum] in PBS + Triton X-100 0,1%, filter the solution before using it.

13 Incubate at least 01:00:00 at Room temperature.

1h

Staining: Day 1

25m

14 Prepare antibody in blocking solution containing 5% NGS.

15 Put a drop (50 µL) of Primary antibody solution on the parafilm surface.

16 Remove the coverslips from the plate and gently put it upside-down on the antibody drop.

17 Incubate Overnight at 4 °C . 1h

Staining: Day 2 25m

18 Wash:

18.1 Wash for 00:05:00 in PBS + Triton X-100 0.1%. (1/3) 5m

18.2 Wash for 00:05:00 in PBS + Triton X-100 0.1%. (2/3) 5m

18.3 Wash for 00:05:00 in PBS + Triton X-100 0.1%. (3/3) 5m

19 Prepare secondary antibody in blocking solution containing 5% NGS.

Note

Note: Keep in the dark.

20 Put a drop (50 µL) of secondary antibody solution on the parafilm.

21 Take the coverslips and put it upside-down on the antibody drop.

22 Incubate 01:00:00 at Room temperature in the dark. 1h

23 Transfer the coverslip to a 24-well containing 500 µL 1X PBS + Triton X-100 0,1%.

24 Incubate for 00:05:00 at Room temperature (in the dark). 5m

25 Dilute DAPI 1:10000 in 1X PBS.

26 Remove PBS and incubate with DAPI for 00:05:00 at Room temperature in the dark. 5m

27 Wash.

27.1 Wash with 1X PBS. (1/2)

27.2 Wash with 1X PBS. (2/2)

28 Mount the slides:

28.1 Put a drop (10 µL) of DAKO mounting reagent on the slide.

28.2 Take out the coverslip from the plate.

28.3 Dry it by gently tapping the coverslip's edge on a lens-cleaner tissue.

28.4 Gently put the coverslips upside-down on the DAKO drop.

28.5 Leave it dry (24:00:00 , DARK, Room temperature). 1d

29 Store in a slide-box at 4 °C .