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# Immunofluorescence staining

Forked from Immunofluorescence staining

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**We use this protocol and it's working**

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## Abstract

This protocol describes the immunofluorescence staining of cells.

## Materials

**Cell culture materials:**
DMEM (Thermo Fisher Scientific, 11965-092)
FBS (Thermo Fisher Scientific, 16140-071)
Penicillin/Streptomicin (10,000 U/mL; Thermo Fisher Scientific, 15140122)
PBS (Thermo Fisher Scientific, 10010023)
Earle’s Balanced Salt Solution (EBSS; Thermo Fisher Scientific, 24010043)

16% Paraformaldehyde (Electron Microscopy Sciences, 15710)
Bovine serum albumin (BSA; Sigma-Aldrich, A9647)
Saponin (Sigma-Aldrich, 47036-50G)
Fluoromount-G (SouthernBiotech, 0100-01)

## Fixation of HEK293 cells in 24 well plate

35m

1 Remove medium and add 4% PFA to the cells.

Note

**Note**: For a coverslip in a 24 well plate use at least 300 µL /well.

2 Incubate 00:20:00 at Room temperature . For cells expressing light sensitive fluorescent proteins, cover the 24 well plate with aluminum foil to block out ambient light.

20m

3 Collect PFA.

4 Perform the following washes with 1× PBS:

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

5m

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

5m

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

5m

## Blocking and permeabilization

15m

5 Remove 1X PBS.

6 Add 300 µL /well of blocking solution.

Note

**Blocking solution**: 5% BSA [Bovine Serum Albumin] in 1× PBS + 0.1% Saponin

7 Incubate at least 00:15:00 at Room temperature . 15m

## Staining: Day 1 25m

8 Prepare primary antibody in blocking solution (typically 1:500, but can vary based on antibody staining).

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

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

11 Alternatively, leave the coverslips in the well and add ( 250 µL ) of the primary antibody solution to each well.

12 Incubate Overnight at 4 °C . 1h

## Staining: Day 2 25m

13 Perform the following washes:

13.1 Wash for 00:05:00 in blocking solution (1/3) 5m

13.2 Wash for 00:05:00 in blocking solution. (2/3) 5m

13.3 Wash for 00:05:00 in blocking solution. (3/3) 5m

14 Prepare secondary antibody in blocking solution (typically 1:500, but can vary based on antibody staining).

Note

**Note**: Keep in the dark. Keep the plate covered in aluminum foil if not doing so previously.

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

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

17 Alternatively, leave the coverslips in the well and add ( 250 µL ) of the secondary antibody solution to each well.

18 Incubate 01:00:00 at Room temperature in the dark (covered in foil). 1h

19 Perform the following washes:

19.1 Wash for 00:05:00 in blocking solution (1/3)

19.2 Wash for 00:05:00 in blocking solution. (2/3)

19.3 Wash for 00:05:00 in blocking solution. (3/3)

20 Mount the slides:

20.1 Put a drop ( 10 µL ) of Fluoromount-G on the slide.

20.2 Take out the coverslip from the plate.

20.3 Dry it by gently tapping the coverslip's edge on a kimwipe.

20.4 Gently put the coverslips upside-down on the Fluoromount-G drop.

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

20.6 Apply clear nail polish around the coverslips to ensure rigidity of the mount.

21 Store in a slide-box at 4 °C .

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