




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Immunofluorescence assay (IFA)

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2 Works for me

 Sharedx.doi.org/10.17504/protocols.io.5qpvobz99l4o/v1 [nguyen.tha](#)

ABSTRACT

This protocol details the procedure of immunofluorescence assay (IFA).

ATTACHMENTS

[ihdnbr7hf.docx](#)

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KEYWORDS

Immunofluorescence, HistoGrip, Permeabilization

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MATERIALS TEXT

Buffers:

- 1x PBS
- **Fixation buffer:** 4% (w/v) paraformaldehyde (PFA) in **0.1 Molarity (M)** phosphate buffer.
- **Permeabilization buffer:** 0.1 TritonX-100/1xPBS
- **Blocking buffer:** 3% Goat serum in 0.1 Tween20/1xPBS

Mounting medium:

A	B
DABCO	0.2M
Tris-Cl pH 8.0	80 mM
Glycerol	90%

 14-Diazabicyclo[2.2.2]octane Sigma-

aldrich Catalog #D27802

- Dissolve **0.23 g** DABCO in **200 µL** H₂O.
- Add **0.8 mL** **1 Molarity (M)** Tris-Cl pH 8.0.
- **9.0 mL** glycerol and mix on rotary shaker.
- Store at **-20 °C** wrapped in foil.

Procedures

2d 6h 35m

- Seed the HeLa cells onto HistoGrip (ThermoFisher) coated coverslips in growth media in 6 well plates (DMEM supplemented with 10 % (v/v) FBS, 1 % Penicillin-Streptomycin, **25 millimolar (mM)** HEPES, GlutaMAX (Life Technologies) and non-essential amino acids (Life Technologies)).

After **48:00:00**, cells can be subjected to any required treatment prior to fixation.

2 

Aspirate off the culture media and add **700 μ L** of fixation buffer to each well and incubate on a side-to-side shaker for **00:10:00**.

3 

Aspirate off the fixation solution and wash three times with 1x PBS.

4 Permeabilise the cells with Permeabilization buffer (**2 mL** for each well) for **00:10:00**^{10m} on a side-to-side shaker.

5 

Aspirate off the Permeabilization buffer and wash once with 1x PBS.

6 

15m

Aspirate off PBS and incubate the cells with Blocking buffer (**700 μ L** for each well) for **00:15:00** on a side-to-side shaker.

7 

Aspirate off the Blocking buffer, wash once with 1x PBS and once with permeabilization buffer.

8 Aspirate off the permeabilization completely and put the 6 well plates on a flat bench.

9 

3h

Add the primary antibodies (diluted to the right concentrations in Blocking buffer; **15 μ L** /well) onto the glass coverslips and incubate for **01:00:00** - **02:00:00** depending on the strength of the antibodies.




10 

Wash the coverslips twice with 1x PBS and once with permeabilization buffer.

11 Aspirate off the permeabilization completely and put the 6 well plates on a flat bench.

12 

3h

Add the relevant secondary antibodies (diluted in Blocking buffer to 1/200-1/500;  **15 µL** /well) onto the glass coverslips and incubate in the dark for  **01:00:00** –  **02:00:00** .


13 

Wash three times with 1x PBS and mount the coverslips on microscope slides.

13.1 Use a yellow tip, drop one small drop off mounting medium onto a microscope slide.

13.2 Pick out one coverslip from a well (still in 1x PBS) and place it onto a piece of whatman paper so that the side that the cells grew on is facing up.

13.3 Take the microscope slide with a drop of mounting medium and put it on the coverslip and gently push it down to remove excessive mounting medium.

13.4 Seal the edges with nail polish, let it dry and store in a microscope box at  **4 °C** prior to imaging.