Jan 16, 2026 Version 1

Immunofluorescence staining –Paraffin sections V.1

DOI
https://dx.doi.org/10.17504/protocols.io.bp2l6ex9rgqe/v1

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Protocol Citation: Domenic Filingeri 2026. Immunofluorescence staining –Paraffin sections. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l6ex9rgqe/v1

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

Created: January 13, 2026

Last Modified: January 16, 2026

Protocol Integer ID: 238596

Keywords: paraffin section, immunofluorescence, dapi step

Abstract

IF staining for paraffin sections (DAPI step included)

Materials

- HistoClear
- Ethanol (100%, 95%, 90%, 80%, 70%, 50%)
- Tap water
- Triton X-100
- PBS
- Sodium citrate buffer (10 mM Sodium citrate, 0.05% Tween 20, pH 6.0)
- EDTA buffer pH 8.0
- Tris-EDTA buffer pH 9.0
- Blocking buffer
- Primary antibody
- Secondary antibody
- 1xDAPI
- Anti-fade mounting medium
- Sodium citrate (dihydrate) 2.94 g
- Distilled water
- 1N HCl
- Tween 20
- EDTA 0.37 g
- NaOH
- Tris 1.21 g
- BSA
- NP40
- Normal Serum

1 60°C for 25 min in dry incubator 25m

2 Re-hydrate with HistoClear and Ethanol, create each as a 50mL conical tube, pour into glass slide box. Pour slowly and not directly onto slides. 45m
HistoClear 10 min X 2 times
100% EtOH 5 min X 2 times
90% EtOH 3 min, (45mL in 5mL DI water)
80% EtOH 3 min, (40mL in 10mL DI water)
70% EtOH 3 min, (35mL in 15mL DI water)
50% EtOH 3 min, (25mL in 25mL DI water)

3 Wash in tap water, 5 min X 3 times 15m

4 **Antigen retrieval**. Select one method from below. Use plastic slide box

4.1 Sodium citrate (pH 2.0, **pH6.0**, or pH 9.0)

4.2 Tris-EDTA buffer pH 9.0

4.3 EDTA buffer pH 8.0

5 Heat them using a presser cooker for 20 minutes in plastic box. Add DI-water to pressure cooker up to metal plate. Select porridge option, remove at "LO 25min", then RT for 1 hour 45m

5.1 Allow to cool to RT slowly. This can take up to 2 hours. (Minimum 30 min) 2h

6 Wash in tap water, 5 min X 2 times 10m

7 Wipe away water, circle section with pap-pen, then add 200uL/slide of tap water. (Use 200uL for all future steps unless noted)

7.1 cut P-200 pipette tip to prevent high pressure for subsequent steps

8 Permeabilize nuclear membrane with 0.25% Triton X-100 in PBS 5 min

5m

9 Wash in tap water 10 min X 2 times

20m

10 Incubate slides with **blocking buffer** for 30min - 1 hour at RT in hydration chamber (add tap water to wells between slides).

1h

10.1 Blocking buffer is 5%BSA in TBST

11 Rinse in PBST briefly

12 Incubate for **primary antibody** diluted in blocking buffer. Leave overnight at 4°C in hydration chamber. (add tap water to wells between slides).

12.1 Antibody diluted in 3%BSA in TBST

13 Wash in PBST 3 min X 3 times. Double wash

10m

14 Incubate with **secondary antibody** diluted in 1x PBS at RT for 30 min to one hour in hydration chamber (dilute in PBS).

1h

14.1 Antibody diluted in 3%BSA in TBST

15 Wash in PBST 3 min X 3 times. Double wash

10m

16 Incubate with 1xDAPI at RT for 15 min in a hydration chamber. (Dilute 1:100 in ddH2O)

15m

17 Wash in PBST 3 min X 3 times. Double wash

10m

18 Add one small drop anti-fade mounting medium (10-15uL) to each section, cover, press with plastic pipette tip to remove bubbles, blot clean with paper towel. Store at least 15', then move to 4∗C

1 mM EDTA, pH 8.0

19 EDTA 0.37 g
Distilled water 1 L
Adjust to pH 8.0 with NaOH
Store at room temperature for 3 months

Tris-EDTA buffer (10 mM Tris base, 1 mM EDTA solution, 0.05% Tween 20, pH 9.0)

20 Tris 1.21 g
EDTA 0.37 g
Distilled water 1 L
Mix to dissolve. Adjust pH to 9.0.
Add 0.5 mL of Tween 20 and mix well. Store at room temperature for 3 months or at 4°C for longer storage.

Blocking buffer

21 10% Normal Serum
1% BSA
0.25% NP40
In 1x PBS

PBST

22 1x PBS
0.1% Tween 20

4x Blocking Buffer stock (50 ml)

23 0.5g BSA in 40 ml of 1xPBS. Mix to dissolve. It can be warmed in a water bath.

Add 1.25 ml of 10% NP40 and mix by inverting. Do not vertexing. It causes a lot of bubbles.
Add 1x PBS up to 50 ml Vol.
Aliquot (250 ul in 1.5 ml tube) and store at –20 C.

Working blocking buffer

24 250ul of 4x blocking buffer
100 ul of normal serum (if all secondary antibodies are from one species)
650 ul of 1x PBS

Sodium Citrate

25 2.94g Tri-Sodium Citrate
800 mL distilled (pure) Water
Mix, dissolve
Adjust pH to 6.0 with 1M HCl
Top off to 200 mL using Pure diH2O
Add 0.5 mL Tween 20, or 5mL 10% Tween

PBST

26 990 mL PBS
10 mL (10% Tween 20) or 1mL (100% tween)

.25%Triton X 100 in PBS

27 10x PBS (900 mL Pure, 100 mL PBS 10X)
2.5 mLTriton X 100
25 mL 10x Triton

Acknowledgements

Edited by Jeong Lee on 6/28/2022