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Immunohistochemistry/Immunofluorescence

In 1 collection

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

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2023

PROTOCOL integer ID:

56552

ABSTRACT

This protocol details about the immunohistochemisty/immunofluorescence staining techniques for tissue.

ATTACHMENTS

338-741.pdf

MATERIALS

Solutions and Reagents

0.5 M Tris (8 L)

A	В	С
Needed (mL)	Stock Solution	Final Concentration
5 L	dH2O	
485 g	Tris base	0.5 M
240 mL	Concentrated HCI	
pH to 7.6		
To 8L	dH2O	

Reagents

A	В	С	D	E
Vendor	Catalog #	Qty	Unit Price	Description
Vector Laboratories	H-3300	1	132.6 0	Antigen Unmasking Solution, Citric Acid Based
Vector Laboratories	H-4000	1	120.0 0	ImmEdge Hydrophobic Barrier Pen

Keywords:

immunohistochemisty, immunofluorescence, formic acid retrieval, Sudan Black Treatment, ASAPCRN

A	В	С	D	E
Vector Laboratories	PK-6100	1	248.6 3	VECTASTAIN Elite ABC Kit (Standard)
Vector Laboratories	SK-4105	1	138.1 3	ImmPACT DAB Peroxidase (HRP) Substrate
Vector Laboratories	BA-2000	1	55	Biotinylated Horse Anti-Mouse IgG Antibody
Vector Laboratories	BA-1100	1	140	Biotinylated Horse Anti-Rabbit IgG Antibody
Sigma	199664- 25G	1	66.60	Sudan Black B
Thermo Fisher	6765001	1	46.41	Shandon Harris Hematoxylin (non acidic)
Fisher Scientific	23-244- 256	1	22.96	Cytoseal 60; 4 oz.
Southern Biotech	0100-01	1	45.14	DAPI Fluoromount-G

- Antigen Unmasking Solution Citrate-Based **Vector**Laboratories Catalog #H-3300
- ImmEdge hydrophobic barrier pap pen Vector

 Laboratories Catalog #H-4000
- VECTASTAIN Elite ABC HRP Kit (Peroxidase, Standard) **Vector**Laboratories Catalog #PK-6100
- ImmPACT® DAB Substrate Peroxidase (HRP) **Vector**Laboratories Catalog #SK-4105
- Horse Anti-Mouse IgG Antibody (H L) Biotinylated **Vector**Laboratories Catalog #BA-2000
- Horse Anti-Rabbit IgG Antibody (H L) Biotinylated **Vector**Laboratories Catalog #BA-1100
- Sudan black B Sigma

 Aldrich Catalog #199664
- Shandon™ Harris Hematoxylin, Nonacidified **Thermo**Fisher Catalog #6765001
- Fluoromount-G Southern
 Biotech Catalog #0100-01

Day 1

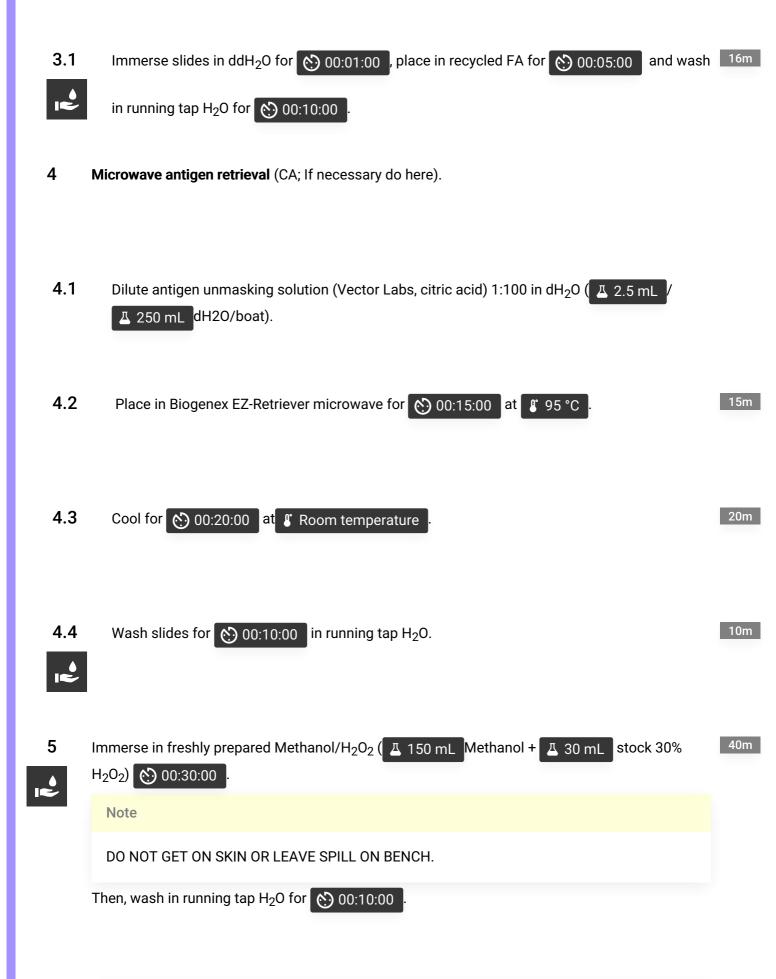
2h 11m

1 Label slides with antibody and treatment to be used.

2 De-paraffinize slides in fresh xylenes, then in a descending ethanol series. 2.1 5m De-paraffinize slides 00:05:00 in fresh xylenes. (1/2) 2.2 5m De-paraffinize slides 00:05:00 in fresh xylenes. (2/2) 2.3 De-paraffinize slides in ethanol 100% for 00:01:00 2.4 De-paraffinize slides in ethanol 100% for (5) 00:01:00 2.5 De-paraffinize slides in ethanol 95% for 00:01:00 2.6 1m De-paraffinize slides in ethanol 80% for 00:01:00 2.7 1m De-paraffinize slides in ethanol 70% for 00:01:00

3

Formic Acid Retrieval (If necessary, do here).



*This step is not necessary for immunofluorescence.

*May use DI water/ H_2O_2 (\bot 150 mL DI water + \bot 50 mL stock 30% H_2O_2).

6 Wash in [M] 0.1 Molarity (M) Tris buffer, [H 7.6] © 00:05:00 Discard all Tris washes.

5m



- 7 Block in [M] 0.1 Molarity (M) Tris/2% FBS (Tris/FBS) 00:05:00 +. Keep blocking solution for up to 2 weeks @ 4 °C.
- **8** Dilute primary antibodies in Tris/FBS, and prepare humidified chamber(s) by soaking towel in the middle of the slide chamber(s).

Note

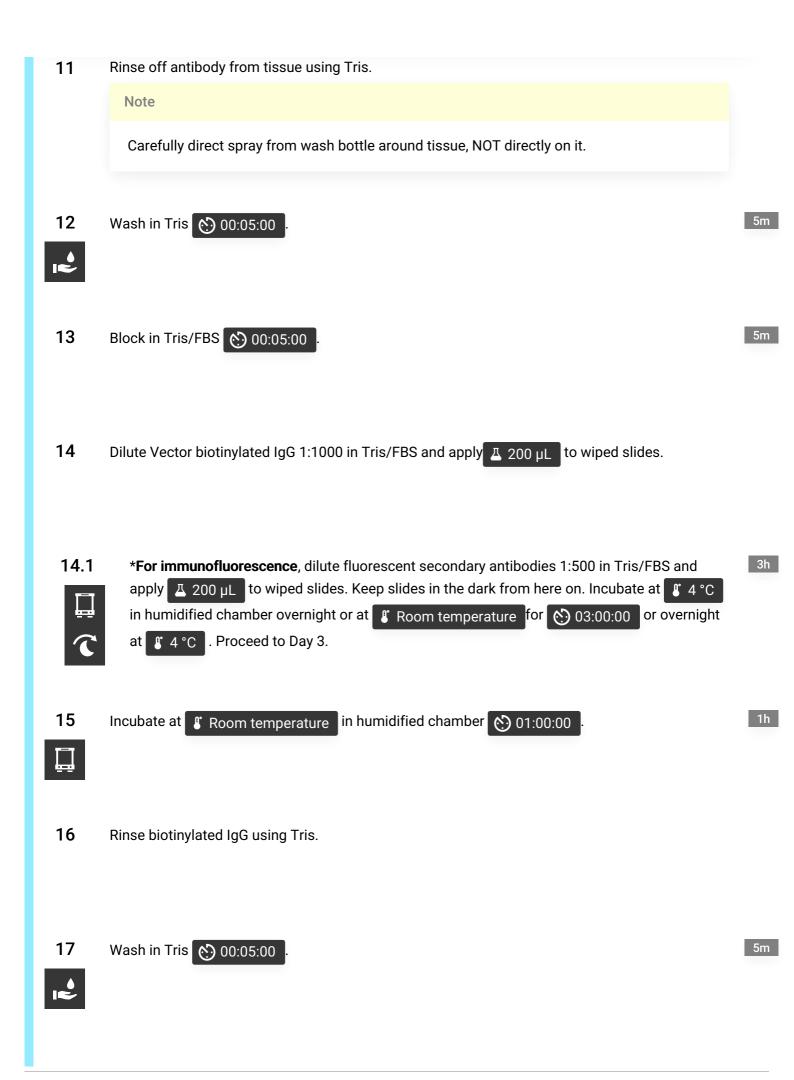
Hydrophobic pen may be used at this point if desired, but CANNOT be used for immunofluorescence.

10 Incubate at [4 °C in humidified chamber Overnight

5m



Day 2



- 21 Rinse off AB using Tris.
- 22 Immerse in Tris (*) 00:05:00
- Make Vector DAB solution (1 drop of DAB per mL of Stable DAB Buffer).
- Apply Z 200 µL of DAB to each slide and incubate until a visible brown signal is seen and well developed.

Note

Development time may differ by antibody, but all sections treated with the same antibody should be developed for the same amount of time.

Rinse with Tris and place in dH_2O . Wash 00:05:00 in dH_2O . Filter Harris hematoxylin.

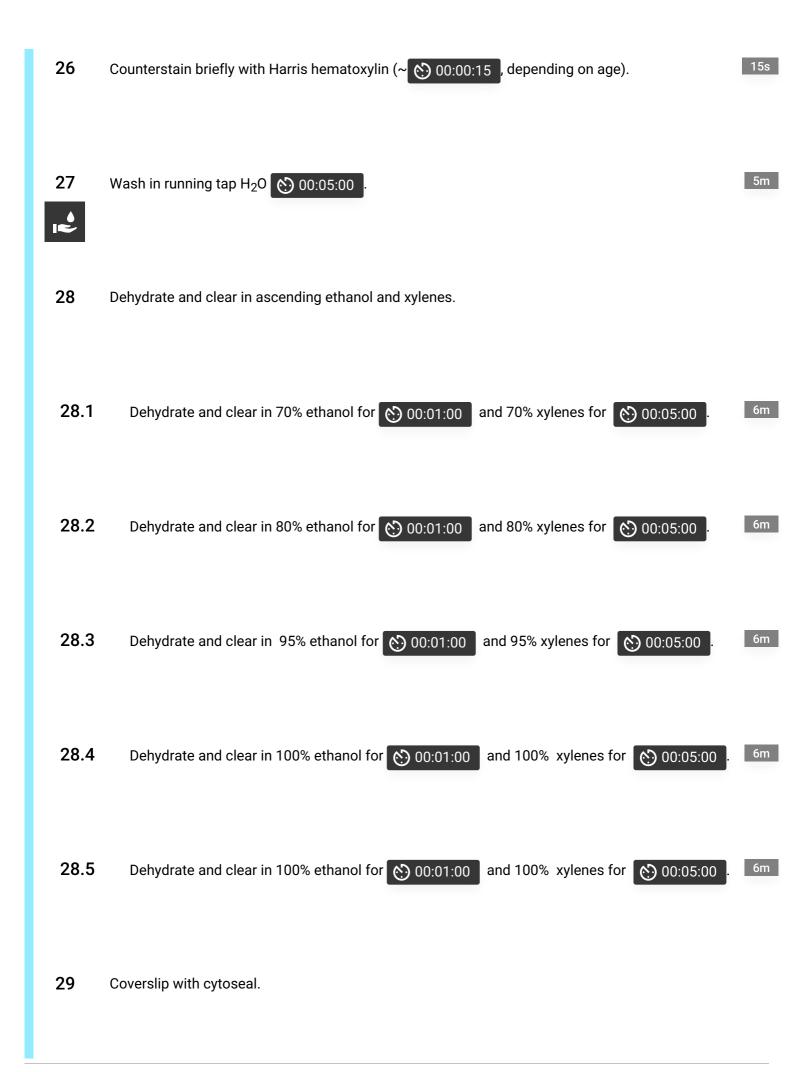


5m

5m

15m

5m





Day 3 (Immunofluorescence)

16m 10s

- 31 Filter Sudan Black. This process can take a long time, so start early.
- **32** Rinse off AB using Tris.
- Wash in running tap H₂O for 00:05:00

5m

- Wash in Tris for 00:05:00 in green boats.

5m

- Ł
- 35 Sudan Black Treatment (0.3% Sudan Black B in 70% Ethanol)
- Use a control slide (usually 1 positive primary and 1 secondary only) to titrate for background 1m 10s reduction without changing signal intensity (usually 00:00:10 to 00:01:00).
- 35.2 Image before and after Sudan black treatment for various times.





Coverslip using non-photobleaching reagent (FluorMount with DAPI). Allow to dry completely before imaging on scanner.