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Immunofluorescence of paraffin-embedded tissue sections

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Carolina Lopez[1]

[1]Washington University

Cecilia Escudero
wustl

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

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Abstract

Protocol for immunostaining of paraffin-embedded tissue sections

Materials

	A	B	C
	Reagent ID	**Vendor**	**Catalog #**
	Antigen Unmasking Solution, Tris-Based (100X)	Vector	H3301
	Antigen Unmasking Solution, Citric Acid Based (100X)	Vector	H-3300
	Xylene	Fisher	X3P-1GAL

Protocol

2h 25m

1 **De-Paraffinization and re-hydration of tissue sections** 30m

For the following incubations, prepare coplin jars or any container that can completely enclosure your slide rack, one per solution. The solutions should completely cover the slides. Between incubations, just move the slide to the next container.

a) Xylene (3 Changes) 00:05:00 each

b) 100% EtOH 00:05:00

c) 95% EtOH 00:05:00

d) 90% EtOH 00:05:00

e) 70% EtOH 00:05:00

f) dH_2O 00:05:00

2 **Antigen Retrieval (if using a steamer)** 1h 20m

1. Preheat steamer in the “cooking” or “boiling” function. Careful to not put a lot of dH2O in the pot so it will overflow to the slide container.
2. Prepare the correct amount of 1x antigen unmasking solution in dH2O and fill a coplin jar or plastic container where the slides will be incubated. Example: 200 mL for a plastic container jar (12 slide rack). Fill up the container, submerge the slide rack and transfer the container + unmasking solution + slide rack to the preheated steamer. Incubate for 00:20:00 at max temperature.
3. Turn of steamer and take off the coplin jar containing the slides. Let it cool down at Room temperature for 01:00:00 .
4. Wash the slides in 1x PBS

OBS.: Troubleshoot the best unmasking solution for your staining. Examples: The Citric Acid Based solution works good for staining Foxp3 in mouse lungs, while Tris-based solution works better for detecting both virus (SARS-CoV-2 N protein) and host cytoplasmic proteins in mouse lungs.

3 **Antigen Retrieval (If using a hot plate)**

1) Place a 2L beaker containing 1X antigen unmasking solution on a hot plate, cover with foil, and turn plate on high for 00:15:00 , then turn low to maintain a mild boil at 102 °C . Check temperature with a thermometer.

2) With forceps, slowly submerge slide rack into mildly-boiling beaker. Boil the slides for 00:20:00 .

3) Turn off the hot plate and let the slides cool down inside the beaker containing antigen unmasking solution for 01:00:00, or until the temperature drops to around 40 °C.

4) Wash slides 3-5 times by moving rack up and down in PBS.

4 **Immunofluorescence Staining**

- Once sections are de-paraffinized, re-hydrated and after antigen retrieval the staining can be performed using the **PROTOCOL FOR TISSUE IFA (**https://www.protocols.io/edit/protocol-for-tissue-ifa-cryopreserved-sections-demm3c46**)** described previously.