Vezina Lab IHC Protocol

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1 Works for me This protocol is published without a DOI.

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PROTOCOL CITATION

Chad Vezina 2020. Vezina Lab IHC Protocol. **protocols.io** https://protocols.io/view/vezina-lab-ihc-protocol-bjv5kn86

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Dewaxing, antigen retrieval and blocking.

1 Heat slides to 65°C for 5 min to remove wrinkles and increase tissue adhesion.

2 Dewax and rehydrate slides by incubating at 25°C in Xylene (3 min, repeat twice), 100% ethanol (3 min, repeat twice), 75% ethanol (3 min, repeat twice) and 50% ethanol (3 min, repeat twice).

3 Prepare a 1X working solution of citrate buffer by adding 5 mL of 100X solution to 495 mL of dH20 and place into a 8”x 8”x 2” Pyrex glass dish.

4 Place slides in glass dish and microwave on power 50 for 20 min. Allow slides to cool to room temperature before proceeding.

5 Use a kimwipe to create a dry rectangle around the tissue section.

6 Outline samples with hydrophobic pen, being careful to mark only the dry part of the slide.

7 Pipette a bead of TBSTw into the outlined region and let sit for 5 min at 25°C with no agitation so that the pap barrier can dry.

8 Tap off TBSTw and block for 1 hr at 25°C with gentle agitation in blocking buffer.

Pause point: samples can be stored for up to 4 hours

9 Dilute primary antibody/antibodies in blocking buffer, apply to slides, and incubate overnight at 4°C with gentle agitation.

Secondary antibody and coverslipping.

10 Wash 5 min in TBSTw at 25°C with gentle agitation (repeat 5 times).

11 Dilute flurophore-conjugated secondary antibody/antibodies in blocking buffer, apply to slides, and incubate for 60 min at 25°C in a light protected box.

12 Remove secondary antibody solution and wash with TBSTw for 5 min at 25°C in light protected box. Repeat this step 7 times.

13 Prepare DAPI working solution by combining 1.5 µl DAPI stock with 998.5 µl TBSTw, apply to slides and incubate for 5 min at 25°C in light protected box.

14 Wash with TBSTw for 5 min at 25°C in light protected box. Repeat this step 3 times.

15 Add a small bead of antifade-mounting media to slide and add cover glass (Corning #2865-18). Image ASAP.

Pause point: Samples can be refrigerated (4ºC) for up to 3 days before imaging without appreciable loss of fluorescence

Solution Preparation

16 **Phosphate Buffered Saline (PBS, 1L of a 1X working solution)**

- Dissolve one packet of Dulbecco's Phosphate Buffered Saline in 1L of H_2O and sterilize by autoclaving.Store at 4°C.

17 **Tris Buffered Saline-Tween-20 (TBSTw, 500 mL of a 10X stock solution)**

- Combine 15.76g Tris-HCl, 43.83g NaCL, 500 mL H_2O, and 5 mL Tween-20 and adjust pH to 7.4. Store at 4°C.

18 **10% (wt/vol) Roche Reagent (100 mL of a 10X stock solution)**

stock solution	final conc.	for 100 mL
maleic acid	100 mM	1.2 g
5 M NaCl	150 mM	3 mL
dH2O to vol.	--	to 100 mL
Blocking reagent	10%	10 g

Mix maleic acid, NaCl & dH_2O according to above, pH to 7.5 (note: this is a strong buffer and it is difficult to adjust pH,

try using solid NaOH pellets to raise pH initially).
Add blocking reagent, microwave briefly to aid solubility.
Aliquot 10 mL volumes into conical tubes & store at –20°C for up to three years.

19 **Citrate Buffer (100x stock solution)**

- 1M Sodium Citrate Dihydrate (Fisher Scientific #BP327-500)
- Dilute with water, adjust pH to 6.0, store at 4°C for up to 6 months

20 **Blocking Solution (100 mL)**

- Combine 10 mL of 10% Roche reagent, 5 mL donkey serum, 1 g BSA Fraction V, and 85 mL TBSTw.Prepare 1 mL aliquots and store at -20°C for up to three years.

21 **Sudan Black B (50 mL)**

- Make a 0.1% stock solution of sudan black by dissolving 50 µg of powder into 50 mL of 70% ethanol.
- Store solution at room temperature
- Make fresh prior to each staining procedure

22 **Mounting Medium (10 mL)**

- Make a n-propyl gallate 20% stock solution by combining 0.4 grams n-propyl gallate and 2 ml DMSO, prepare 100 microliter aliquots and store at -20°C
- Make mounting medium by combining 0.5 mL of 20X PBS solution, 9 mL glycerol, 100 microliters of 20% n-propyl gallate, and 0.5 mL H_2O. Store solution at 4°C for up to 2 months.

23 **4',6-diamidino-2-phenylindole, dilactate (DAPI) solution (10 mL of a 300 µM/ 10X stock solution).**

- Combine 0.0014 g DAPI and 10 mL N,N Dimethylformamide. Prepare 100 µl aliquots and store in dark at -20°C for up to 1 year.