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## Immunohistochemistry- mounted slides

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1 Works for me

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### 1 Day 1

- Ø Using the PAP Pen, carefully draw a water barrier circle around the tissue sections on the slide – allow this circle to dry for several seconds or up to approx. one min.
- Ø Rinse slides with PBS (pH 7.3-7.4): 4 x 5 min each
- Ø Rinse slides with 0.5% BSA + 0.4% Triton X-100 in PBS: 1 x 10 min
- Ø Remove slides one at a time and using a clean Kimwipe, carefully wipe around the tissue sections to dry the slide.
- Ø Place the slides into a black, covered slide incubation box/humidity box
- Ø Cover the tissue sections with blocking buffer (10% normal donkey serum in 1.0% BSA + 0.4% Triton X-100 + PBS)
- Ø Allow the sections to remain in blocking buffer for 1.5-2 hrs. at RT
- Ø Pour off the blocking buffer
- Ø Replace with primary antibody solution (antibody of choice diluted in 1.0% BSA + 0.4% Triton X-100 + PBS)
- Ø Incubate tissue with primary antibody overnight in incubation box.

### 2 Day 2

- 3 Ø Rinse slides with PBS: 4 x 5 min
- Ø Rinse slides with 0.5% BSA + 0.4% Triton X-100 + PBS: 1 x 10 min
- Ø Prepare fluorescent secondary antibody (secondary antibody should be diluted in 1.0% BSA + 0.4% Triton X-100 + PBS) (*use Biotin-SP for the antibody that will later add Streptavidin to; if there is a second primary then use the normal secondary for it*).
- Ø Cover the tissue with the secondary antibody solution and incubate for 2 hrs. at RT in the incubation box. \*From this point on, use low light and/or cover tissues.\*
- Ø Rinse slides with PBS: 4 x 5 min
- Ø Remove excess PBS with a Kimwipe.
- Ø Prepare fluorescent Streptavidin secondary antibody (should be diluted in 1.0% BSA + 0.4% Triton X-100 + PBS)
- Ø Cover the tissue with the fluorescent Streptavidin antibody solution and incubate for 2 hrs. at RT in the incubation box.
- Ø Rinse slides with PBS: 4 x 5 min
- Ø Remove excess PBS with a Kimwipe.
- Ø Carefully add a drop of Citifluor (or equivalent) mounting medium to the center of the tissue and apply cover glass.
- Ø Seal cover glass with clear nail polish. For thicker tissue, add a weight before sealing.



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