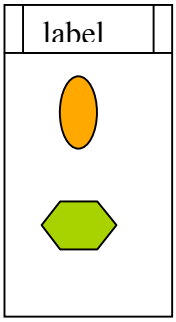
Immunohistochemistry basic plan:

Slides should have sections expected to be positive and expected negative on same slide

Frozen sections
---Air dry, freeze
or use today or tomorrow



Paraffin sections
See —different protocol below

---Make washing buffer: PBS or PBST (PBS with 0.1% Tween
---Make Blocking buffer: 0.5% fish gelatin in washing buffer
Or 1% BSA in washing buffer
---Set up humid chamber using 250 ml of washing buffer

---Fixation needed?
---Endogenous peroxidase removal if using HRP system
---Endogenous biotin removal if using biotin labels

Set 1
Unfixed
overlay with blocking buffer
Remove endogenous
Peroxidase,Biotin

Set 2
Acetone fixed (Fisher cat# A16-4)
10 minutes at room temperature
Wash immediately in buffer
Overlay with blocking buffer
Remove endogenousPeroxidase/Biotin

Set 3 ----------fix later
Overlay with blocking buffer
Remove endogenousPeroxidase/Biotin
---Fix by immersion in 10% NBF
(Fisher cat# SF93-4)
--for 30 minutes in fume hood
at room temperature
--wash in washing buffer

Overlay slides, one at-a-time
in humid chamber with
--1--blocking buffer
--2--non-specific IgG
--3--positive control Ig
--4--primary antibody at dilution 1
--5--primary antibody at dilution 2
--6--primary antibody at dilution 3
--7--Pre-immune at dilution 1
--8--Pre-immune at dilution 2
--9--Pre-immune at dilution 3

Incubate primary reagents in humid chamber for specified amount of time, followed by 3 buffer washes

Incubate with secondary reagent,
Followed by 3 buffer washes
Incubate with tertiary if needed
Followed by 3 buffer washes
Substrate and nuclear counterstain if needed

Paraffin sections: deparaffinize, rehydrate and wash with buffer
Divide into 5 sets, to test optimal conditions—may do this in 2 batches
--set 1--Untreated,
--set 2--Proteinase K treated,
--set 3--Heat in citrate buffer pH 6,
--set 4--Heat in Tris buffer pH 9
--set 5-- Heat in EDTA buffer pH 8

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Based on results from first couple of trials, the epitope in the section may need to be revealed with an extra enhancement using the Biotinyl tyramide reagent