

Date: **Immunohistochemistry checks**

Aim:

Tissue sections: Frozen Paraffin

---Frozen Acetone fixation: A. Acetone 10 minutes, → Immediate PBS washes

---Blocking:

-endogenous peroxidase: 0.03% OR 0.3% H₂O₂ in PBS for 20 minutes,
followed by PBS washes

-endogenous biotin: 0.1% avidin 10 minutes, wash;
0.01% biotin 10 minutes, wash

---Frozen sections Fix in 4% fresh paraformaldehyde
or 10% buffered formalin

Paraffin: Deparaffinization

Antigen retrieval: Yes No

a) 0.1% trypsin or trypsinEDTA

b) heat in 0.1M citrate, cool 20 minutes

c) heat in 1mM EDTA pH 8, cool 20 mins

d) 0.01% TritonX100, PBS washes

---Block endogenous collagen binding: 1% BSA/PBS;--- ? add 10% serum

---Primary reagents: Dilutions time incubation

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____

-----Wash in buffer x3

---Secondary reagents Dilutions time incubation

-----Wash in buffer x3

---Tertiary reagents: Dilutions time incubation

-----Wash in buffer x3

-----Further tiers and enhancements?:

-----Chromagen? :

-----Wash in buffer x3

-----Mayers hematoxylin/Nuclear fast red:

-----Wash in buffer x3

-----Aquamount