

## NB400-127 Protocol

### Immunohistochemistry-paraffin embedded sections protocol (NB400-127)

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#### Immunohistochemistry-paraffin embedded sections

##### Antigen Unmasking

- Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0 then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top for 30 minutes.

##### Staining

- Wash sections in dH<sub>2</sub>O three times for 5 minutes each.
- Wash section in wash buffer (1X PBS/0.1% Tween-20 (1X PBST)) for 5 minutes.
- Block each section with 100-400 ul blocking solution (1X PBST, 5% goat serum) for 1 hour at room temperature.
- Remove blocking solution and add 100-400 ul primary antibody diluted in 1X PBST, 5% goat serum to each section. Incubate overnight at 4C.
- Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
- Add 100-400 ul biotinylated secondary antibody, diluted in 1X PBST, 5% goat serum. Incubate 30 minutes at room temperature.
- Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
- Add 100-400 ul Streptavidin reagent to each section and incubate for 30 minutes at room temperature.
- Wash sections three times in wash buffer for 5 minutes each.
- Add 100-400 ul DAB substrate to each section and monitor staining closely.
- As soon as the sections develop, immerse slides in dH<sub>2</sub>O.
- Counterstain sections in hematoxylin.
- Wash sections in dH<sub>2</sub>O two times for 5 minutes each.
- Dehydrate sections.
- Mount coverslips.