

NB400-127 Protocol

Immunohistochemistry-Paraffin protocol for Niemann-Pick type C1 Like-1 Antibody (NB400-127)

[[URL:https://www.novusbio.com/products/niemann-pick-type-c1-like-1-antibody_n...]][[Caption:Niemann-Pick type C1 Like-1 Antibody]]

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 dH₂O 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 dH₂O.
- Counterstain sections in hematoxylin.
- Wash sections in dH₂O two times for 5 minutes each.
- Dehydrate sections.
- Mount coverslips.