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NB100-2527 Protocol

Immunohistochemistry-paraffin embedded sections

LOX Antibody: https://www.novusbio.com/products/lox-antibody_nb100-2527
Antigen unmasking

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

Staining

1. Wash sections in dH2O three times for 5 minutes each.
2. Wash section in wash buffer (1X PBS/0.1% Tween-20 (1X PBST)) for 5 minutes.
3. Block each section with 100-400ul blocking solution (1X PBST, 5% goat serum) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul primary antibody diluted in 1X PBST, 5% goat serum to each section.
5. Incubate overnight at 4C.
6. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
7. Add 100-400 ul biotinylated secondary antibody, diluted in 1X PBST, 5% goat serum.
8. Incubate 30 minutes at room temperature.
9. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
10. Add 100-400 ul Streptavidin HRP reagent to each section and incubate for 30 minutes at room temperature.
11. Wash sections three times in wash buffer for 5 minutes each.
12. Add 100-400 ul DAB substrate to each section and monitor staining closely.
13. As soon as the sections develop, immerse slides in dH2O.
14. Counterstain sections in hematoxylin.
15. Wash sections in dH2O two times for 5 minutes each.
16. Dehydrate sections.
17. Mount coverslips.