

NB110-3638 Protocol

Immunohistochemistry-Paraffin Protocol for Alkaline Phosphatase, Placental Antibody (8B6) (NB110-3638)

Alkaline Phosphatase/ALPP Antibody (8B6): https://www.novusbio.com/products/alkaline-phosphatase-alpp-antibody-8b6_nb110-3638

Antigen Unmasking - Proteolytic Induced Epitope Retrieval (PIER):

Trypsin Working Solution (0.05%):

Trypsin stock solution (0.5%) -1 ml

Calcium chloride stock solution 1% - 1 ml Distilled Water - 8 ml Adjust pH to 7.8 with 1N NaOH.

Cover sections with trypsin working solution and incubate for 10-20 minutes at 37 degrees Celsius in humidified chamber (optimal incubation time may vary depending on tissue type and degree of fixation, and should be determined by user). Allow sections to cool at room temperature for 10 minutes.

Staining:

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