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## NBP2-53421 Protocol

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Immunohistochemistry-Paraffin Protocol for Lactate Dehydrogenase B Antibody (NBP2-53421)

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 (keep slides in the sodium citrate buffer at all times).

## Staining:

- 1. Wash sections in deionized water three times for 5 minutes each.
- Wash sections in PBS for 5 minutes.
- 3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) 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 HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
- 7. Wash sections three times in wash buffer for 5 minutes each.
- 8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
- 9. As soon as the sections develop, immerse slides in deionized water.
- 10. Counterstain sections in hematoxylin.
- 11. Wash sections in deionized water two times for 5 minutes each.
- 12. Dehydrate sections.
- 13. Mount coverslips.