

NB200-179 Protocol

Immunohistochemistry paraffin-embedded protocol (NB200-179)

[[URL:https://www.novusbio.com/products/15-pgdh-hpgd-antibody_nb200-179]][[Caption:15-PGDH/HPGD Antibody]]

Immunohistochemistry

1. 5 μ M-thick formalin-fixed paraffin-embedded tissue sections were baked at 60C for 75 minutes, deparaffinized, and rehydrated.
2. Antigen retrieval was performed by steaming the sections at 96C for 5 minutes in 10 mM citrate buffer (pH 6.0), plus a cool-down period of 20 minutes.
3. Reduction of peroxidases was accomplished by incubating in 3% H₂O₂ in water for 30 minutes at room temperature.
4. Avidin ??biotin blocking was performed for 15 minutes each, followed by nonspecific protein blocking (Serum-Free Protein Block, Dako, Carpenteria, CA) performed for 60 minutes.
5. Primary antibody was diluted in 1% BSA and incubated overnight at 4C in humidified chambers.
6. The slides were washed thoroughly, and Protein Block was added again for 30 minutes.
7. LSAB+ anti-rabbit kit (Dako) was used for development, applying the secondary antibody and HRP-conjugated streptavidin per the manufacturer's instructions.
8. Diaminobenzidine (Dako) was added to the slides for 10 minutes.
9. The sections were then counterstained by using Harris modified hematoxylin stain (Fisher Scientific) for 1 minute.

**Note: All washes were done with TBS (50 mM Tris-HCl/150 mM NaCl, pH 7.6) diluted in deionized water.