Orders: orders@novusbio.com
Support: technical@novusbio.com
Web: www.novusbio.com
Protocols, Publications, Related Products, Reviews and more:
www.novusbio.com/NB200-114

NB200-114 Protocol

IHC (Paraffin) Protocol Specific for MMP2 Antibody (NB200-114)

[[URL:https://www.novusbio.com/products/mmp-2-antibody-8b4_nb200-114]][[Caption:MMP-2 Antibody (8B4)]]
For IHC on Paraffin-Embedded Tissue Sections:
1. Deparaffinize in xylene. 3 changes at 10 min each.
2. Hydrate in 100% EtOH for 5 min (2X).
3. Quench endogenous peroxidase. 30 min in 1% H2O2 in methanol. (Make fresh each time: 6 ml 30% H2O2+ 194 ml methanol).
4. Hydrate to H2O in graded alcohols.
a. 5 min in 95%.
b. 5 min in 70%
c. 5 min in distilled H2O.
5. Perform antigen retrieval (Antigen Retrieval; Dako #S1700) with heating as described by manufacturer. Fill plastic Coplin jar with 50 ml Antigen Retrieval buffer. Add slides to jar when temperature reaches 95-99C start timing. Heat for 30 min. After heating, let slides cool in jar for 15 min.
6. Wash slides in PBS for 5 min (2x).
7. Infiltrate sections with PBS + 0.5% Triton X100 for 10 min, then rinse twice in PBS.
8. Block for 1 hr. at room temp in Blocking buffer- (PBS + 10% goat serum).
9. Aspirate blocking buffer. Apply MMP2/8B4 IgG diluted to 5 ug/ml in Blocking buffer. Incubate overnight at 4C (or >2 hr at 37C).
10. Rinse 3X in PBS for 10 min each.
11. Apply 2 degrees antibody. Dilute biotin conjugated goat anti-mouse 1:500 (Dako # E0433) in Blocking buffer.
12. Incubate for 2 hr. at room temperature.
13. Rinse 3X in PBS for 10 min each.
14. Prepare Avidin-biotin-peroxidase by combining each component at 1:50 in PBS + 0.1% Triton X100. Gently mix and react for 30 min. Prior to use, dilute the complexed mixture 1:5 with PBS + 0.1% BSA. Apply to sections and incubate for 2 hr at room temp.
15. Rinse in 3X in PBS for 15 min each.
16. Develop with DAB. Dissolved 1.5 mg diaminobenzidine-(HCl)4 in 3 ml of PBS, then add 2 ul of 30% hydrogen peroxide. Filter through a 0.2 um filter immediately before use. Develop for 15 min.
17. Rinse in water for 5 min. Counterstain for nuclei (e.g., hematoxylin) as desired.
18. Dehydrate through graded ethanol, clear in 100% ethanol and xylene (1:1 solution) and then 100% xylene. Coverslip with Permount.