

STAINING PROTOCOL for paraffin-embedded tissue-sections fixed in formalin

Anti-human (rat) Megalin (#CD7D5)

Ultra Vision LP Large Volume Detection System HRP Polymer (Ready-To- Use)
Fa. Thermo scientific
Nr. TL-125-HL

1. Deparaffinize through xylenes, ethanol, and hydrate to water.
2. Pre-treatment: in digestive enzyme: Protease XIV (4,5units = 86mg/70ml) in Tris-Puffer at 37°C 10 min
3. (Sigma)/TBS 10min at 37°C
4. Block in 3% hydrogen peroxide in TBS 10 min
5. Wash in buffer
6. Apply Ultra V Block and incubate 5 min at room temperatur
7. Wash
8. Apply Primary Antibody **CD7D5** at a dilution 1:500(liquid), 1:1000(Lyophil) in 1%BSA/PBS for 1 hour
9. Wash 4 times
10. Apply Primary Antibody Enhancer and incubate for 10 min at room temperatur
11. Wash 4 times
12. Apply HRP Polymer and incubate for 15 min at room temperatur in the dark(HRP Polymer is light sensitive)
13. Wash 4 times
14. Incubate with ACE-Chromogen and stain for 5- 10 min
15. Wash 4 times in AD
16. Counterstain with Mayer'sHämalaun for 1 min
17. Coverslip using an aqueous mounting media