

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

**Anti-human Podocalyxin (#GB6D9C11)**

Vectastain ABC Standard Kit  
Nr. TL-125-HL

1. Deparaffinize through xylenes, ethanol, and hydrate to water.
2. Heat-Pre-treatment: in 0,1mol citratbuffer pH 6 in an Autoclave at 1bar for 10 min
3. Cool down at roomtemperatur for 20 min
4. Block in 3% hydrogen peroxide in PBS 10 min
5. Wash in buffer
6. Block with Avidin-block for 10 min
7. Wash
8. Block with Biotin-block for 10 min
9. Wash
10. Block with 10% normal goatserum
11. Apply Primary Antibody: **GB6D9** at a dilution 1:100 in 10%GS/PBS for 1 hour
12. Wash 4 times
13. Apply Biotinylated goat anti mouse (Vector) 1:300 and incubate for 30 min
14. Add 1 drop of A and one drop of B to 5 ml buffer, mix it and incubate 30 min
15. Wash 4 times
16. Apply the ABC- mix for 30 – 45 min
17. Wash 4 times
18. Incubate with ACE-Chromogen and stain for 5- 10 min
19. Wash 4 times in AD
20. Counterstain with Mayer's Hämalaun for 1 min
21. Coverslip using an aqueous mounting media