

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

**Anti-rat podoplanin (#LF3 (B7)D5B3)**

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. Heat-Pre-treatment: in 0,1mol citratbuffer pH 6 in an Autoclave at 1bar for 10 min
3. Cool down at room temperature for 20 min
4. Block in 3% hydrogen peroxide in PBS 10 min
5. Wash in buffer
6. Apply Ultra V Block and incubate 5 min at room temperature
7. Wash
8. Apply Primary Antibody: **LF3 (B7)D5B3** at a dilution 1:1000 in 1% BSA/PBS for 1 hour
9. Wash 4 times
10. Apply Primary Antibody Enhancer and incubate for 10 min at room temperature
11. Wash 4 times
12. Apply HRP Polymer and incubate for 15 min at room temperature 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's Hämalaun for 1 min
17. Coverslip using an aqueous mounting media