

## **Anti-human Podoplanin (Cat# 101-M40) for formalin-fixed paraffin-embedded sections**

- 1) Put Paraffin sections (2-4  $\mu\text{m}$ ) onto a Poly-Lysin object holder
- 2) Deparaffination:
  - Xylol (10 min)
  - Acetone (10 min)
  - Acetone / buffer mixture (10 min)
  - Rinse 3x with wash buffer
- 3) Microwave: boil up 3x 10 min in the appropriate buffer (put the whole glass curette into the microwave)
  - a) EDTA buffer: 0,1 M EDTA in aqua dest, pH 8,0
  - b) Citrate buffer/10 mM, pH 6,0
    - A: citric acid, 19,2 g in 1L aqua dest
    - B: Na-Citrate, 29,41 g in 1L aqua dest

9 ml stock solution A + 50 ml stock solution B, in 500 ml aqua dest (pH adjustment only in solution B)

  - Cool down the sections to RT for 25 min in washing buffer  
**(for CRLR-Ab: Citrate buffer is the better solution!)**
- 4) Addition of primary ABs (dilute in ready to use buffer from DAKO [S3022])
  - Shortly Drip off the sections on paper (take 3-4 sections at once)
  - Dust off the liquid beside the sections
  - Add primary ABs
  - incubate 0.5 h at RT
  - Rinse 3x with wash buffer
  - Shortly Drip off the sections
- 5) Addition of the by-pass AB (monoclonal mouse anti-rabbit IgG)
  - Code-Nr. M 0737 1: 250
  - incubate 0.5 h at RT
  - Rinse 3x with wash buffer
  - Shortly Drip off the sections
- 6) Addition of anti-mouse-IgG from rabbit 1:
  - Incubate 0.5 h at RT
  - Rinse 3x with wash buffer
  - Shortly Drip off the sections
- 7) Addition of the APAAP complexes
  - Incubate 0.5 h at RT
  - Rinse 3x with wash buffer
  - Shortly Drip off the sections
- 8) Addition of the Chromogens (NaphtolAS-MXPhosphat plus FastredTR-Salz)
  - Incubate for 25 min at RT
  - Rinse 3x with wash buffer
  - Shortly Drip off the sections

**(6,7,8: kit from DAKO: Universal-DAKO APAAP Kit Maus)**

  - Counterstain: Hämalaun

- 45 sec
- rinse 3x 5 min in lukewarm tap water
- rinse 1x 5 min in dest. H<sub>2</sub>O

9) Cover the sections in Glycergel (DAKO)

Note: After each step the sections could stay in the buffer!

**Wasch buffer:** (approach for 40 L)

351g NaCl  
274g Tris-HCL  
36g Tris-Base