

Deparaffinization: slides with paraffined sections should be treated as follows:

- 6 min xylene 1
- 6 min xylene 2
- 6 min 100% ETOH
- 6 min xylene 2
- 6 min 100% ETOH
- 6 min 95% ETOH
- 6 min 80% ETOH
- 6 min 50% ETOH
- Wash with distilled water
- 5 min in 1X TRIS or in PBS
- 1X **RETRIEVAL** for 1 min at 450°C (to expose the epitopes contained in the tissue that remained masked during fixation)
- 1X **RETRIEVAL** for 5 min at 100°C (open the microwave in case the solution boils to avoid the detachment of the section)
- Bring to room temperature for 30 min
- Wash with distilled water
- 5 min in 1X TRIS or in PBS
- **Permeabilize** in a 0.1% Triton X-100 solution (diluted in PBS) for 10 min
- 5 min in 1X TRIS or in PBS
- 3 min in 3%-diluted H₂O₂
- Wash with distilled water
- 5 min in 1X TRIS or in PBS
- 20 min of **BLOCKING** (human serum AB diluted 1:10 in PBS)
- Incubate with primary antibody overnight (dilute in Dako Diluent)
- Bring to room temperature for 30 min
- 5 min in 1X TRIS or in PBS

Autor: M. Todaro

01/02/2015

Approved: Giorgio Stassi



IMMUNOSTAINING

- 30 min BIOTINYLATE (except for goat)
- 5 min in 1X TRIS or in PBS
- 30 min STREPTAVIDINA (except for the goat)
- 5 min in 1X TRIS or in PBS
- AEC Chromogen (= AminoEtilCarabazole) for approximately 15 minutes (check the development)
- Dip in distilled water
- 5 min in distilled water
- 2 min in non-alcoholic 1:2-diluted haematoxylin (the diluted solution should be filtered with filters 45 to avoid precipitations)
- Dip in distilled water
- Dip 3 times in **ammonia water (2‰)** to contrast the colored nuclei
- Dip in distilled water
- Proceed with the mounting

Autor: M. Todaro

01/02/2015

Approved: Giorgio Stassi

