

## Deparaffinization:

- 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** (pH 6.1) for 1 min at 450°C (to expose the epitopes contained in the tissue that remained masked during fixation)
- 1X **RETRIEVAL** (pH 6.1) 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 PBS
- **Permeabilize** in a 0.1% Triton X-100 solution in PBS for 10 min
- 5 min in PBS
- For positive control: treat with 50 µl 1:50-PBS-diluted DNase for 10 min at room temperature
- 5 min in PBS
- Incubate for 1 hour at 37°C in the 100 µl reaction mixture of TUNEL (mix composed of buffer + enzyme, diluted 1:10)
- Bring to room temperature for 30 min
- 5 min in PBS



## TUNEL

- Incubate for 30 min at 37°C with an anti-fluorescein antibody conjugated with alkaline phosphatase (Converter -AP).
- 5 min in PBS
- Add the chromogenic solution BCIP / NBT (5-bromo, 4-chloro, 3-indolylphosphate / Nitro-Blue Tetrazolium) and incubate for 10 minutes at room temperature
- Wash with distilled water
- Proceed with the mounting

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