IHC deparaffinization protocol
Overview

This step is required when using paraffin embedded sections. Before proceeding with the staining protocol, the slides must be de-paraffinized and rehydrated. Incomplete removal of paraffin can lead to poor staining of the section.

Materials and reagents

- Xylene
- 100% ethanol
- 95% ethanol
- 70% ethanol
- 50% ethanol

Method

Place the slides in a rack, and perform the following washes:

1. Xylene: 2 x 3 min
2. Xylene 1:1 with 100% ethanol: 3 min
3. 100% ethanol: 2 x 3 min
4. 95% ethanol: 3 min
5. 70% ethanol: 3 min
6. 50% ethanol: 3 min
7. Running cold tap water to rinse

Keep the slides in the tap water until ready to perform antigen retrieval. At no time from this point onwards should the slides be allowed to dry. Drying out will cause non-specific antibody binding and therefore high background staining.