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Immunohistochemistry Protocol for Formalin Fixed Paraffin Embedded (FFPE) Tissues

1. Deparaffinization and rehydration of sections

- Heat the slides 30 - 40 min in 62 - 65°C (Immunostainer, heating plate, or oven)
- Deparaffinate the sections in xylene and rehydrate sections in graded ethanol (Immunostainer or manually)

2. Heat Induced Epitope Retrieval (HIER)

- Immerse the slides in desired antigen retrieval solution, eg. TRIS-EDTA pH9 HIER or Citrate pH6 HIER
  - PT-module: +98°C with 20 min treatment
  - Micro-oven: 2 x 10 min with 400-600w (Try to avoid boiling)

- Allow to cool down. If HIER performed separately (not in the immunostainer), avoid to dry the warm slides! Drying of the slides affects staining intensity significantly. Immerse slides as soon as possible into the TBS-TWEEN washing buffer.

3. Staining

- After HIER:

1.	Rinse slides in TBS-tween	
2.	Primary antibody	20 - 30 min
3.	Wash with TBS-Tween	
4.	3 % H2O2	5- 10 min
5.	Wash	
6.	Detection reagent with HRP label	30 min
7.	Wash with TBS-Tween	
8.	DAB (BCB-20032)	10 min
9.	Aqua	
10.	CuSO4 -post enhancement (Optional)	5-10 min
11.	Aqua	
12.	Counter staining in diluted Mayer (1:5)	2 min
13.	Bluing in tap water	5-10 min
14.	Dehydration in graded ethanol, clearing in xylene and mounted with xylene based permanent mounting media	

Dilution of the concentrated antibody depends on the detection system used and the final working dilution need to always be determined by the user.

The protocol is provided by BioSiteHisto´s IHC experts who has developed the Optibodies™ – for diagnostic and research purposes.