IHC-P: Staining Protocol - Chromogenic Detection

Important: Some proteins have special requirements for good detection. Please refer to the **remarks** sections for IHC-P on the respective data sheet.

Tissue preparation

For the preparation of paraffin embedded tissues for immunohistochemistry, please refer to our **tissue preparation protocols.**

Materials and reagents

- **Food steamer** (e.g. Braun Multigourmet; alternatively: microwave, water bath, pressure cooker)*
- **Staining containers with slide holders** (e.g. Tissue-Tek)
- **Blocking buffer:** Protein Block Serum Free (Agilent cat. no. X0909)
- **Antibody incubation buffer:** Antibody diluent (Agilent cat. no. S2022)
- **Biotinylated secondary antibody**
- **ABC HRP Kit:** standard (Vectorlabs cat. no. PK-4000)
- **ImmPACT DAB:** (Vectorlabs cat. no. SK-4105)
- **PBS:** Phosphate buffered saline, (pH 7.4)
- **TBST:** Tris buffered saline with Tween 20, 50 mM Tris (pH 7.6), 150 mM NaCl, 0.05% Tween 20
- **Antigen retrieval buffer:** 10 mM citrate, 0.05% Tween 20, pH 6.0 or 10 mM Tris, 1 mM EDTA, 0.05% Tween 20, pH 9.0. Please check IHC-P remarks on the respective data sheet.
- Xylene, 100% ethanol, 90% ethanol, 80% ethanol and 70% ethanol, 2-propanol
- **Optional:** Hematoxylin Solution (Mayer's, Modified) or other nuclear counterstain
- **Optional:** Avidin/Biotin Blocking Kit (Vectorlabs cat. no. SP-2001)
- **Non-aqueous mounting medium**

Deparaffinization and rehydration

Deparaffinize and hydrate tissue sections

1. Xylene 2x 5 min
2. 100% EtOH 2x 2 min
3. 90% EtOH 1x 2 min
4. 80% EtOH 1x 2 min
5. 70% EtOH 2x 2 min
6. Deionized Water 1x 20 sec
7. PBS 1x 2 min

Keep the slides in PBS until ready to perform the Antigen Retrieval. Do not allow the slides to dry out.

Antigen retrieval (using a food steamer)*

1. Heat the steamer with a suitable staining container filled with **Antigen retrieval buffer** to **~97°C**.
2. Transfer the sections into the staining box, wait until the temperature reaches **97°C.**
3. Incubate the sections in the steamer for **30 min.**
4. Remove the staining container from the steamer and allow the slides to cool down for **20 min** (target end temperature **~60°C**).

Blocking

1. Wash slides in PBS, 3x 1 min.
2. Incubate the sections with 3% hydrogen peroxide in PBS (freshly prepared!) for **5 min** to block endogenous peroxidase activity.
3. Wash slides in PBS, 2x 1 min.
4. Wash slides in TBST, 1x 2 min.
5. **Optional:** Some antibodies require an additional antigen retrieval step with **formic acid.** Please check IHC-P remarks on the respective data- or factsheet. If formic acid treatment is required, incubate slides for **3 min** in **88% formic acid**. Wash slides in TBST, 3x 1 min.
6. **Optional:** Perform Avidin-Biotin-Block according to manufacturer's instructions.
   Note: Certain tissues (e.g. liver, kidney) contain high levels of endogenous biotin. The Avidin-Biotin blocking step is recommended when using the ABC system for these tissues. If the background problem persists, consider trying a polymer-based detection system instead of biotinylated secondary antibody/ABC system.
7. Block in **blocking buffer** for **10 min.**

Antibody incubation

1. Drain slides (do not rinse).
2. Apply primary antibody diluted in **antibody incubation buffer** and incubate in a humidified chamber for **1 h at room temperature.**
3. Wash slides in TBST, 3x 2 min.
4. Apply secondary antibody diluted in **antibody incubation buffer** for 30 min at room temperature.
5. In the meantime, prepare the ABC-reagent: 5 ml PBS + 1 drop A + 1 drop B and incubate for 30 min.
6. Apply the ABC reagent for 30 min at room temperature.
7. Wash slides in TBST, 3x 2 min.

Chromogenic detection with DAB

1. Apply the **DAB substrate** for 1-10 min.
   Note: Observe the staining with a microscope! Development times may differ depending upon the level of antigen.
2. Stop the DAB reaction with deionized water.

Counterstain (optional)

1. Follow the manufacturer's instructions for counterstaining and bluing.
2. Wash slides in deionized water for 1 min.

Dehydration and mounting

1. 70% EtOH 2x 10 sec
2. 80% EtOH 1x 10 sec
3. 90% EtOH 1x 10 sec
4. 2-Propanol 3x 1 min
5. Xylene 3x 2 min

Mount slides in a suitable organic mounting medium and add coverslip.

**For an alternative Antigen Retrieval protocol using a water bath check protocol-ihc-paraffin-fluorescent.*

Note: The SYSY standard protocol generates good staining results in the SYSY labs and may be used as suggestion. However, to achieve the highest specific signal and lowest non-specific background signal, the best antigen retrieval condition, antibody concentration, incubation temperature and incubation time must be determined individually.