

## 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.