

# Protocol: Immunohistochemistry

The Prestige Antibodies® are developed for immunohistochemistry based expression profiling and each Prestige Antibody is accompanied by 576 human tissue immunohistochemistry (IHC) images on the Human Protein Atlas web portal ([www.proteinatlas.org](http://www.proteinatlas.org)). The IHC staining is performed using a standard protocol as described below.

## Immunohistochemistry procedure

### **Deparaffinization**

Paraffin sections of 4µm thickness are baked overnight at 50°C. Prior to immunostaining, deparaffinization and hydration is done in xylene and graded ethanol to distilled water. During hydration, a 5 minutes blocking for endogeneous peroxidase is done in 0.03 % H<sub>2</sub>O<sub>2</sub> in 95 % ethanol.

### **Standard Antigen Retrieval Method**

The standard antigen retrieval method is Heat Induced Epitope Retrieval (HIER) in retrieval buffer pH 6, using a pressure boiler (Decloaking chamber, Biocare Medical, Walnut Creek, CA, USA) as heat source.

HIER is performed by heating the TMA-slides immersed in retrieval buffer for 4 minutes at 125°C in the pressure boiler. After completed boiling, slides remain in the pressure boiler and are allowed to cool to 90°C. The total processing time is approximately 45 minutes.

The standard primary antibody dilution is based on the primary antibody stock concentration:

- dilution 1:25 for primary antibody stock concentration of <0.06 mg/ml
- dilution 1:75 for primary antibody stock concentration of >0.06 mg/ml
- dilution 1:150 for primary antibody stock concentration of >0.1 mg/ml

**NOTE:** The specified working dilutions of the primary antibodies are to be considered as a guideline only. Optimal dilutions must be determined by the user.

### **Immunohistochemical staining program, Autostainer 480®**

(Lab Vision, Fremont, CA, USA)

All incubations are done at room temperature

1. Rinse in wash buffer
2. Incubation with primary antibody for 30 minutes
3. Rinse in wash buffer (x2)
4. Incubation with labeled polymer for 30 minutes
5. Rinse in wash buffer (x2)
6. Developing in DAB solution for 10 minutes

7. Rinse in distilled water (x2)
8. Counterstaining in hematoxylin for 5 minutes\*\*
9. Rinse in tap water for 5 minutes\*\*
10. Rinse in lithium carbonate water, diluted 1:5 from saturated solution for 1 minute\*\*
11. Rinse in tap water for 5 minutes\*\*
12. Dehydration in graded ethanol and xylene\*\*
13. Coverslipping\*\*

All reagents are applied at a volume of 300 µl per slide.

\*\* Steps 8-13 are done in a histostaining instrument (Leica Autostainer XL).

### **Reagents**

For immunohistochemistry, the following reagents are commercially available from Lab Vision, Fremont, CA, USA:

- Wash buffer (10x concentrate). Working solution originally contains 0.05 % (v/v) Tween 20. Extra Tween 20 is added to a final concentration of 0.20 %
- Retrieval Solution: Citrate buffer<sup>®</sup>, pH 6
- Antibody diluent
- UltraVision LP HRP polymer<sup>®</sup> and DAB plus substrate system<sup>®</sup>

In addition, Mayer's hematoxylin (Sigma-Aldrich, St. Louis, MO, USA) is required.

### **Alternative Antigen Retrieval Method**

For selected Prestige Antibodies, alternative retrieval buffers and/or enzymatic antigen retrieval may have been used as stated on the Certificate of Analysis and on the Antigen/Antibody information page on the Human Protein Atlas website.

#### *Enzymatic Antigen retrieval*

Enzymatic retrieval is performed in the immunostaining instrument and refers to incubation of TMA-slides in Proteinase K (Lab Vision, Fremont, CA, USA) for 10 minutes at room temperature.

#### *Heat Induced Epitope Retrieval (HIER) in retrieval buffer pH 9*

HIER in retrieval buffer pH 9 is performed as the standard HIER except that retrieval buffer with pH 9 (Lab Vision, Fremont, CA, USA) is used instead of retrieval buffer with pH 6.