# Standard IHC Protocol

**Atlas Antibodies standard immunohistochemistry protocol**

Described is the Atlas Antibodies standard immunohistochemistry protocol optimized for Triple A Polyclonals and PrecisA Monoclonals.

## Deparaffinization

Paraffin sections of 4 μm thickness are baked overnight at 50°C. Prior to immunostaining, deparaffinization and hydration are performed in xylene and graded ethanol to distilled water. During hydration, we perform 5 minutes blocking for endogenous peroxidase in 0.3% $H_2O_2$ in 95% ethanol.

## Antigen retrieval

### 1. Standard antigen retrieval method

The standard antigen retrieval method is Heat Induced Epitope Retrieval (HIER) in retrieval buffer pH 6,1, using a pressure boiler (Decloaking chamber, Biocare Medical, Walnut Creek, CA, USA) as a heat source. HIER is performed by heating the TMA-slides immersed in retrieval buffer for 20 minutes at 110°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 60 minutes.
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.

### 2. Alternative antigen retrieval methods

For selected antibodies, alternative retrieval buffers and/or enzymatic antigen retrieval may have been used as stated in the Product Datasheet and on the Antibody/ Antigen information page on the Human Protein Atlas.

**Enzymatic antigen retrieval method**

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

### Heat-Induced Epitope Retrieval (HIER) in Retrieval Buffer pH 9

HIER is performed in Target Retrieval solution pH 9 (Dako, Agilent, Santa Clara, CA, USA) using Decloaking Chamber NxGen as described above.

## Immunohistochemical staining program

### Autostainer 480 staining program

(ThermoFisher Scientific, Waltham, MA, USA)

All incubations are performed at room temperature.
All reagents are applied at a volume of 300 µl per slide.

1. Rinse in wash buffer.*
2. Incubation with Ultra V Block for 5 min.
3. Rinse in wash buffer (x2).
4. Incubation with primary antibody for 30 min.
5. Rinse in wash buffer (x3).
6. Incubation with Primary Antibody Enhancer for 20 min.**
7. Rinse in wash buffer (x2).
8. Incubation with labeled polymer for 30 min.
9. Rinse in wash buffer (x2).
10. Developing in DAB solution for 5 min.
11. Rinse in distilled water.
12. Counterstaining in hematoxylin for 5 min.***
13. Rinse in tap water for 5 min.
14. Rinse in lithium carbonate (Li2CO3) water, diluted 1:5 from saturated solution for 1 min.
15. Rinse in tap water for 5 min.
16. Dehydration in graded ethanol and xylene.
17. Mount in Pertex.
18. Coverslip.

* Steps 1 – 11 are performed in Autostainer 480S (ThermoFisher Scientific, Waltham, MA, USA).
** For polyclonal antibodies skip steps 6 and 7.
** Steps 12 -18 are performed in fully automated integrated stainer Leica ST5010-CV5030 (Leica Biosystems Nussloch GmbH, Nussloch, Germany).

## Reagents

For immunohistochemistry, the following reagents are commercially available from Thermo Fisher Scientific, Waltham, MA, 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%.
- Antibody diluent

- UltraVision LP HRP polymer®
- Primary Antibody Enhancer (only for monoclonal antibodies)
- Ultra V Block
- DAB Quanto Detection System (including chromogen and substrate)

For epitope retrieval, the following buffers are commercially available from Dako, Agilent, Santa Clara, CA, USA:

- Target Retrieval Solution, Citrate pH 6.1 (10x)
- Target Retrieval Solution, pH 9 (10x)

In addition, Mayer's hematoxylin and xylene (Histolab, Gothenburg, Sweden) are used.