IHC PROTOCOL

IHC standard protocol optimized for Triple A Polyclonals and PrecisA Monoclonals from Atlas Antibodies.

DEPARAFFINIZATION

Paraffin sections of 4 µm thickness are baked overnight at 50°C. Prior to immunostaining, deparaffinization and hydration is performed in xylene and graded ethanol to distilled water. During hydration, a 5 minutes blocking for endogenous peroxidase is performed 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 (Dako, Agilent, Santa Clara, CA, USA) using a pressure boiler (Decloaking chamber NxGen, Biocare Medical, Pacheco, CA, USA) as 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 completing the 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 method

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

Enzymatic antigen retrieval method

Enzymatic retrieval is performed in the immunostaining instrument by incubating TMA-slides in Proteinase K solution (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

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. Incubate with Ultra V Block for 5 minutes.
3. Rinse in wash buffer (x2).
4. Incubate with primary antibody for 30 minutes.
5. Rinse in wash buffer (x3).
6. Incubate with Primary Antibody Enhancer for 20 minutes.**
7. Rinse in wash buffer (x2).
8. Incubate with labeled polymer for 30 minutes.
9. Rinse in wash buffer (x2).
10. Develop in DAB solution for 5 minutes.
11. Rinse in distilled water.
12. Counterstain in hematoxylin for 5 minutes.***
13. Rinse in tap water for 5 minutes.
14. Rinse in lithium carbonate (Li_2CO_3) water, diluted 1:5 from saturated solution for 1 minute.
15. Rinse in tap water for 5 minutes.
16. Dehydrate 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).

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REAGENTS

For immunohistochemistry, the following reagents are commercially available from Thermo Fisher Scientific, Waltham, MA, USA:

1. 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%.
2. Antibody diluent
3. UltraVision LP HRP polymer®
4. Primary Antibody Enhancer (only for monoclonal
5. antibodies)
6. Ultra V Block
7. 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.

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