Immunohistochemical protocol

All IHC staining in the Human Protein Atlas project is performed using a standard protocol as described below.

Deparaffinization

Paraffin sections of 4 µm thickness (cut using a water fall microtome) are dried at RT overnight and then baked 12-24 h at 50°C. Prior to immunostaining, deparaffinization and hydration is done in xylene and graded ethanol to distilled water. During hydration, a 5 min blocking for endogeneous peroxidase is done in 0.3% H_2O_2 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 min 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 min.

Immunohistochemical staining program, Autostainer 480®

(ThermoFisher scientific, Runcorn, UK)
All incubations are done at RT.
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 labeled HRP polymer for 30 min.
7. Rinse in wash buffer (x2).
8. Developing in DAB solution for 5 min.
9. Rinse in distilled water.
10. Counterstaining in hematoxylin for 7.5 min.**
11. Rinse in tap water for 5 min.**
12. Rinse in lithium carbonate water, diluted 1:5 from saturated solution for 1 min.**
13. Rinse in tap water for 5 min.**
14. Dehydration in graded ethanol and Neo-Clear.**
15. Coversliping.**

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

** Steps 10-15 are done in Autostainer XL® (Leica biosystems, Vista, CA, USA)

Reagents

For immunohistochemistry, the following reagents are commercially available from Thermo scientific, Lab Vision Corporation, Freemont, 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®, pH 6.
• Antibody diluent.
• UltraVision LP HRP polymer®, Ultra V Block and DAB quanto substrate system®.

In addition, Mayer's hematoxylin plus (Histolab, Västra Frölunda, Sweden) is used, as well as Neo-Clear® (VWR, Radnor, PA, USA).

The primary antibody dilution is based on titration optimization, the dilution suggested by the Human Protein Atlas can be found under antibody and antigen information for each antibody.
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.*

Alternative secondary antibody

When primary antibody originates from other host animals than rabbit, one additional step is included (between 7 and 8) and different secondary antibody is used.

Alternative Antigen Retrieval Method

For selected antibodies, alternative retrieval buffers and/or enzymatic antigen retrieval may have been used as stated on the Antigen/Antibody information page on the Human Protein Atlas.
Enzymatic Antigen retrieval
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 min at RT.
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 pH is 9 instead of 6 (Lab Vision, Freemont, CA).