

Immunohistochemistry Protocol

This protocol has been supplied by the Human Protein Atlas (HPA).

Reagents List

For immunohistochemistry, the following reagents are commercially available from DakoCytomation, Glostrup, Denmark, Lab Vision, Fremont, CA, USA, Sigma, St. Louis, MO, USA, and Histolab, Gothenburg, Sweden.

Reagent	Details
SEQer™ antibodies	Please visit our website: www.antibodies.sdix.com for details.
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% (v/v)
Retrieval Solution	Target retrieval solution®, pH6 (Dako Cytomation)
Retrieval Solution	Citrate buffer®, pH6 (Lab Vision)
Retrieval Solution:	Target retrieval solution®, pH9 (Dako Cytomation)
Antibody diluent	Dako Cytomation or Lab Vision
Proteinase K	Dako Cytomation or Lab Vision
ChemMate Envision/DAB®	A kit that contains Ready to use EnVision HRP polymer and chromogen: substrate (Dako Cytomation)
UltraVision LP HRP polymer® and DAB plus substrate system®	Lab Vision
Harris hematoxylin and Destaining solution (Concentrated HCl and 95% ethanol, diluted 1:99)*	Sigma
Mayer's hematoxylin*	Histolab

* Harris hematoxylin and destaining solution (concentrated HCl and 95% ethanol, diluted 1:99), or Mayer's hematoxylin (Histolab) are required.

Deparaffinization

Paraffin sections of 4µm thickness are baked overnight at 37°C, followed by 30 minutes at 60°C. Prior to immunostaining, deparaffinization and hydration is done in xylene and graded ethanol to distilled water. During hydration, a 5 minutes blocking for endogenous peroxidase is done in 0.03% H₂O₂ in 100% ethanol.

Immunohistochemistry Procedures

Antigen retrieval

The test procedure includes immunostaining with and without antigen retrieval. When necessary, two alternative antigen retrieval techniques are used:

Heat Induced Epitope Retrieval (HIER): HIER is performed by heating the TMA-slides immersed in retrieval solution, either at pH6 or pH9 at 125°C for 4 minutes in a pressure boiler (Decloaking chamber, Biocare medical). After completed boiling, slides remain in the pressure boiler and are allowed to cool down to 90°C. The total processing time is approximately 45 minutes.

Enzymatic Antigen Retrieval: Enzymatic retrieval is performed by incubation of the TMA-slides in Proteinase K for 10 minutes at room temperature (RT). If no conclusive results are obtained in the first test round, further tests are performed based on the initial test results.

Immunohistochemical staining

Recommendations and guidelines are based on initial tests using Autostainer 480 instruments® (Lab Vision). The main steps are outlined below:

- 1.0** Primary antibodies and a peroxidase conjugated dextran polymer visualization system are incubated for 30 minutes each at RT.
- 2.0** Slides are developed using Diaminobenzidine (DAB) as chromogen.
- 3.0** Counterstaining is done manually in either Harris or Mayer's hematoxylin.

Antibody dilution:

For SEQer™ antibodies SDI recommended a 1:1000 dilution as starting point.

NOTE: The recommended dilution range is 1:250 – 1:2,000 based on 1 mg/ml of antibody solution.

For technical support, please email antibodysupport@sdix.com
or call 1.800.481.9737.