ATLAS ANTIBODIES

Immunohistochemistry in Human Tissues

Immunohistochemistry and the Human Protein Atlas

Immunohistochemistry (IHC) is the most widely used technique in histopathological diagnosis and research for detection of proteins in tissues and cells. Today, IHC can be applied in a high-throughput fashion for studying proteins by the use of Tissue Microarrays (TMAs). In the Human Protein Atlas project, Triple A Polyclonals are used to analyze all human proteins using IHC and TMAs[1,2]. All resulting tissue and cell images are publicly available on the Human Protein Atlas web portal (proteinatlas.org)[3,4]. In total, more than 500 high resolution IHC images from human tissue samples are presented for each antibody.

The 14th version of the Human Protein Atlas, released in April 2015, presents a tissue-based map of the complete human proteome. The extensive amount of data is divided into four separate 'sub atlases': the Tissue Atlas, the Cancer Atlas, the Subcell Atlas and the Cell Line Atlas. For all proteins represented in the Tissue Atlas, the expression profiles are based on IHC analysis on a large number of human tissues. The presentation of protein expression data in correlation to RNA sequencing data for each gene has now been included. In the Cancer Atlas, differentially expressed genes in several cancers can be studied, while the Subcell Atlas presents subcellular localization by confocal microscopy. Additional information about protein expression in common cell lines is included in the Cell Line Atlas, which has become an appreciated toolbox for research.

Tissue Microarrays

The TMA technology provides an automated array-based high-throughput technique in which as many as 1,000 paraffin-embedded tissue samples can be brought into one paraffin block in an array format. This allows for protein expression profiling in large scale. Each antibody in the Human Protein Atlas project generates more than 500 high-resolution images corresponding to normal and cancer tissues. In this manner, an IHC atlas for tissue expression and localization is built up for each protein, divided into a Tissue Atlas and a Cancer Atlas. TMAs used within the Human Protein Atlas project include samples from up to 44 different human normal tissue types and 20 different types of cancer. Normal tissues are sampled from 144 different individuals and cancer tissues are derived from 216 unique tumors[1,2].

Tissue Microarray Production

TMAs are constructed by extracting cylinders of formalin fixed, paraffin embedded tissue from donor blocks with a sharp punch and assembling them into a recipient block with properly sized holes in a grid pattern[5] (Figure 1). From one array block, approximately 250 sections can be achieved and prepared for IHC analysis.

IHC method in the Human Protein Atlas Project

Within the Human Protein Atlas project, antibody production and analysis are performed in a high-throughput fashion[3,4]. Therefore the immunohistochemistry procedure is highly automated and performed under standardized conditions. As antigen retrieval, Heat Induced Epitope Retrieveal (HIER) is performed in citrate buffer at pH 6, using a pressure boiler. The antibodies are diluted using a dilution robot and staining is performed in an Autostainer. A Horse Radish Peroxidase (HRP)-conjugated polymer together with the chromogen diaminobenzidine (DAB) are used for detection. The specific binding of an antibody to its corresponding antigen results in a brown staining (Figure 2). The tissue section is counterstained with hematoxylin. Hematoxylin staining is unspecific and results in a blue coloring of both cells and extracellular material.

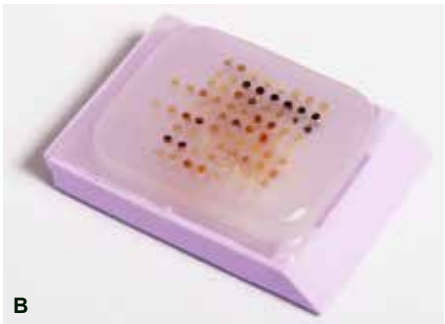


Figure 1.
Cylinders from donor blocks are extracted and inserted into a recipient block. **A**) Donor blocks of formalin fixed, paraffin embedded human tissues. **B**) Recipient block (Tissue Microarray) representing 44 different human normal tissue types ready to be sectioned and used for IHC analysis.

AAA POLYCLONALS
Designed by researchers, for researchers

The antibodies developed and characterized within the Human Protein Atlas project are made available to the scientific community by Atlas Antibodies under the brand name Triple A Polyclonals.

PrecisA Monoclonals are developed by Atlas Antibodies, based on the knowledge from the Human Protein Atlas with careful antigen design and extended validation of antibody performance. With precise epitope information following all monoclonals, these precise, accurate and targeted antibodies are denoted PrecisA Monoclonals.

PrecisA MONOCLONALS
DESIGNED BY RESEARCHERS FOR RESEARCHERS

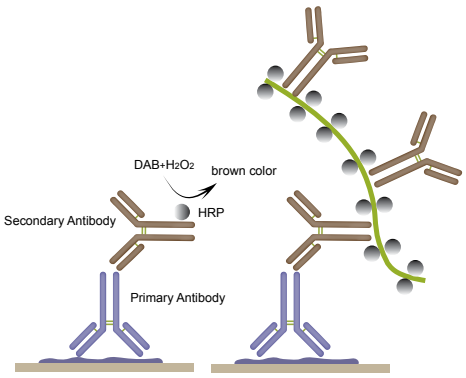


Figure 2.
Schematic figure of the immunohistochemical staining reaction. Triple A Polyclonals and PrecisA Monoclonals are used as primary antibodies and the secondary antibody is labeled with the enzyme HRP. HRP forms a complex with the substrate H_2O_2 and in the presence of the chromogen DAB, a brown color can be visualized using light microscopy. The signal can be amplified using an enzyme-linked dextran polymer (figure to the right).

Antibody Approval

Trained professionals determine the optimal dilution and approve antibodies based on a comparison of staining pattern, available information from gene and protein public databases, as well as in-house technical validation such as protein arrays, RNA sequencing information and Western Blots.

Image Annotation

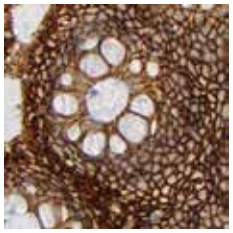
All immunostained slides are scanned to generate high-resolution images. The images representing immunostained tissue sections are analyzed and annotated manually by trained pathologists. All images and annotations are published and freely available at the Human Protein Atlas portal (proteinatlas.org).

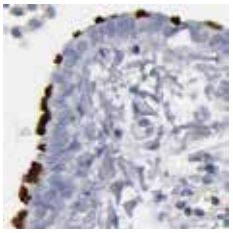
Subcellular analysis using IHC

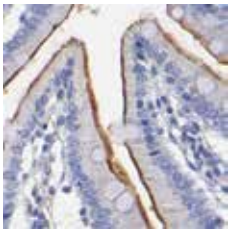
Data on where proteins are localized within a cell provides important information as to what basic functions a protein may have as well as a possibility to map possible other interacting proteins.

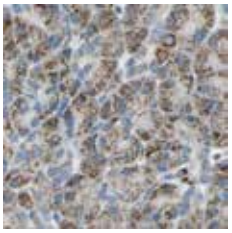
Immunofluorescence (IF)-based imaging is the established golden standard for visualization of proteins at a subcellular level. The vast majority of studies based on immunofluorescence are performed on cultured cells though, with the disadvantage of not being able to analyze cells in their natural tissue context.

Figure 3 shows that also using immunohistochemistry, localization information at a subcellular level can be achieved. Figure 3 A-C show examples of immunohistochemical stainings for recognition of cell membrane-related proteins, Figure 3 D-F show examples of proteins expressed in different cytoplasmic compartments and Figure 3 G-I show proteins expressed in different nuclear structures.

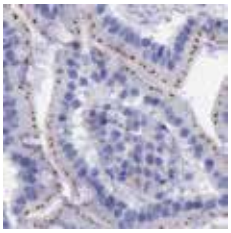

A. Plasma Membrane, Anti-CDH1 (HPA004812), rectum

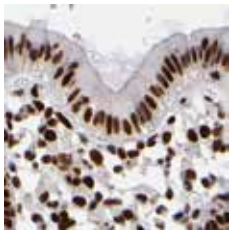

B. Cilia, Anti-ODF2 (HPA001874), fallopian tube

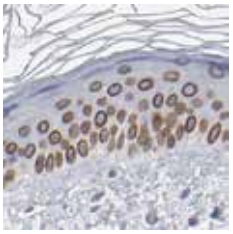

C. Microvilli, Anti-SLC44A2 (HPA003228), small intestine


D. Mitochondria, Anti-ACADM (HPA006198), pancreas


E. Golgi, Anti-GOLGA5 (HPA000992), gall bladder


F. Lysosomes, Anti-GLA (HPA000966), duodenum


G. Nucleus, Anti-MRE11A (HPA002691), colon


H. Nuclear membrane, Anti-SYNE2 (HPA003435), skin

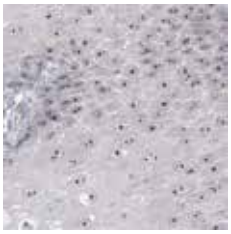

I. Nucleoli, Anti-NOP16 (HPA036506), esophagus

Figure 3.
IHC stainings showing the subcellular location of different proteins. Recognition of target antigen is represented by brown color.

Summary

- The use of Triple A Polyclonals in IHC on Tissue Microarrays (TMAs) has allowed for protein expression profiling in a large-scale format.

- In the Human Protein Atlas project, TMAs including samples from up to 44 different human normal tissue types and 20 different types of cancer are used for protein localization analysis.

- For each antibody, more than 500 IHC tissue images are publicly available on the Human Protein Atlas web portal proteinatlas.org.

- By the use of Triple A Polyclonals and PrecisA Monoclonals in immunohistochemistry studies, information on a subcellular level can be achieved.

References:

1) Pontén F *et al.* The Human Protein Atlas - a tool for pathology. *J Pathology* 2008 216(4):387-93.

2) Kampf C *et al.* Antibody-based tissue profiling as a tool in clinical proteomics. *Clin Proteomics* 2004 1(3-4):285-300.

3) Uhlén M *et al* Towards a knowledge-based Human Protein Atlas. *Nat Biotechnol* 2010 28(12):1248-50.

4) Berglund L *et al.* A gene-centric human protein atlas for expression profiles based on antibodies. *Molecular & Cellular Proteomics* 2008 7:2019-2027.

5) Kampf C *et al.* Production of tissue microarrays, immunohistochemistry staining and digitalization within the human protein atlas. *J Vis Exp.* 2012 May 31;(63).

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