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 using Tissue Microarrays (TMAs). In the Human Protein Atlas project, Triple A Polyclonal antibodies have been designed 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 Human Protein Atlas project has created a complete map of protein expression in all major organs and tissues in the human body[1,2]. To accomplish this, highly specific antibodies directed against all of the human proteins were generated and subsequent protein profiling was established in a multitude of tissues and cells. The Human Protein Atlas (www.proteinatlas.org) consists of three separate parts, each using a particular approach to study the spatial distribution of human proteins; the Tissue Atlas showing the distribution of the proteins across all major tissues and organs in the human body, the Cell Atlas showing the subcellular localization of proteins in single cells, and finally the Pathology Atlas showing the impact of protein levels for survival of patients with cancer.

The Tissue and Pathology Atlases

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 for each protein, divided into a Tissue Atlas and a Pathology Atlas. Samples from up to 44 different human normal tissue types and 20 different types of cancer have been used. Normal tissues are sampled from 144 different individuals and cancer tissues are derived from 216 unique tumors[3,4].

IHC method in the Human Protein Atlas Project

Within the Human Protein Atlas project, antibody production and analysis is performed in a high-throughput fashion[6], with the immunohistochemistry procedure highly automated and performed under standardized conditions.

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.

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 each array block, approximately 250 sections can be achieved and prepared for IHC analysis.

Antigen Retrieval and Staining

For 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 combination of a secondary antibody and a 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.

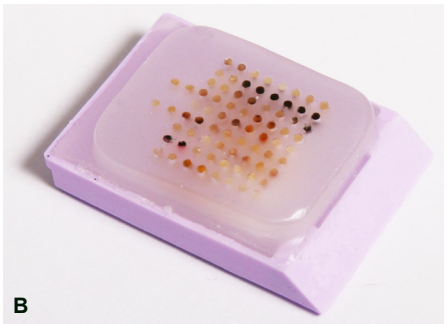


Figure 1.
Cylinders from donor blocks are extracted and inserted into a recipient block. **A**) Donor blocks of formalin fixed, paraffin embedded human tissues. **B**) Recipient block (Tissue Microarray) representing 44 different human normal tissue types ready to be sectioned and used for IHC analysis.

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, these precise, accurate and targeted antibodies are denoted PrecisA Monoclonals.

PrecisA MONOCLONALS
DESIGNED BY RESEARCHERS FOR RESEARCHERS

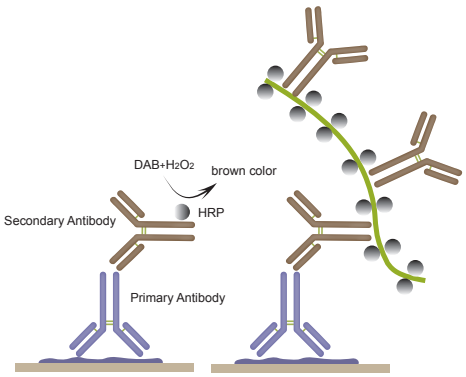


Figure 2.
Schematic figure of the immunohistochemical staining reaction. Triple A Polyclonals and PrecisA Monoclonals are used as primary antibodies and the secondary antibody is labeled with the enzyme HRP. HRP forms a complex with the substrate H_2O_2 and in the presence of the chromogen DAB, a brown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

Evaluation and Validation

Antibody Approval

The optimal dilution is determined and the antibodies are approved based on a comparison of staining pattern, available information from gene and protein public databases, as well as inhouse technical validation such as protein arrays, RNA sequencing information and Western Blots.

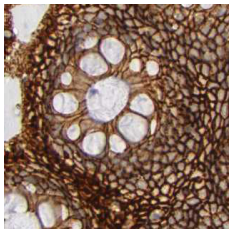
Image Annotation

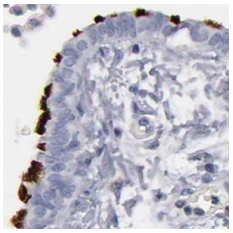
All immunostained slides are scanned to generate high-resolution images. The images representing immunostained tissue sections are analyzed and annotated manually by trained pathologists. All images and annotations are published and freely available at the Human Protein Atlas portal (proteinatlas.org).

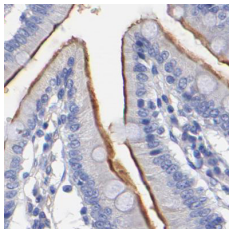
Subcellular Analysis Using IHC

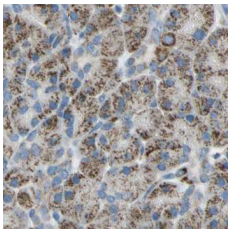
Data on the localization of proteins within a cell provides important information as to what basic functions a protein may have as well as a possibility to map possible other interacting proteins. The established golden standard for visualizing proteins at a subcellular level is immunocytochemistry-immunofluorescence (ICC-IF). The vast majority of studies based on ICC-IF are performed on cultured cells though, with the disadvantage of not being able to analyze cells in their natural tissue context.

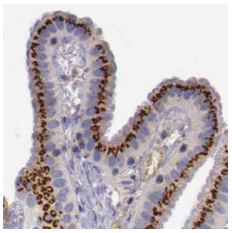
Figure 3 shows that also immunohistochemistry can be used to localize proteins at a subcellular level. Figure 3 A-C shows examples of immunohistochemical stainings for recognition of cell membrane-related proteins. Figure 3 D-F shows examples of proteins expressed in different cytoplasmic compartments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

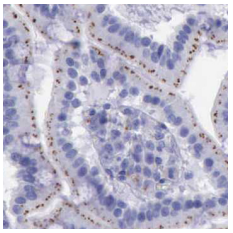

A. Plasma Membrane, Anti-CDH1 (HPA004812), rectum

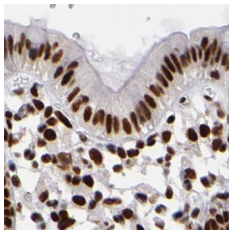

B. Cilia, Anti-ODF2 (HPA001874), fallopian tube

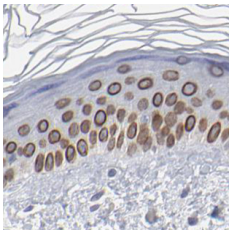

C. Microvilli, Anti-SLC44A2 (HPA003228), small intestine


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

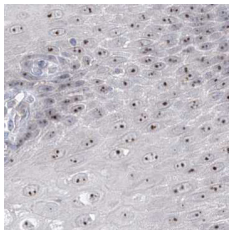

I. Nucleoli, Anti-NOP16 (HPA036506), esophagus

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

Summary

- The use of polyclonal antibodies 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 antibodies in immunohistochemistry studies, information even on a subcellular level can be achieved

References:

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

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

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).

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

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