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 six separate parts, each using a particular approach to study the spatial distribution of human proteins; the Tissue Atlas, the Cell Atlas, the Pathology Atlas , the Brain Atlas, the Blood Atlas showing the impact of protein levels for survival of patients with cancer and the Single Cell Type Atlas.

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.

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 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
- Information studies, even on a subcellular level, is easily achieved using antibodies in IHC.

Figure 1. Tissue Microarrays in IHC
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.

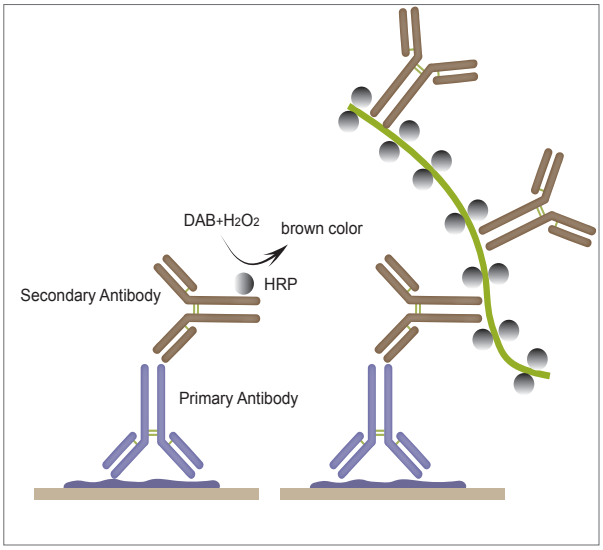


Figure 2. Schematic figure of the immunohistochemical staining reaction.
Triple A Polyclonals and PrecisA Monoclonals are used as primary antibodies. The secondary antibody is labeled with the enzyme HRP which forms a complex with the substrate H_2O_2. In the presence of the chromogen DAB, a brown color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

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).

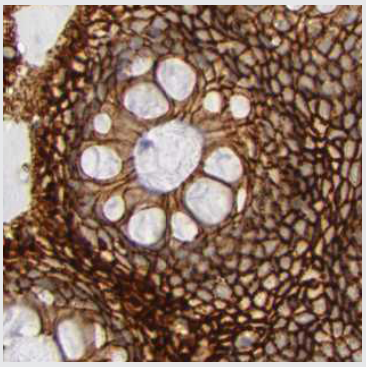
Subcellular Analysis Using IHC

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.

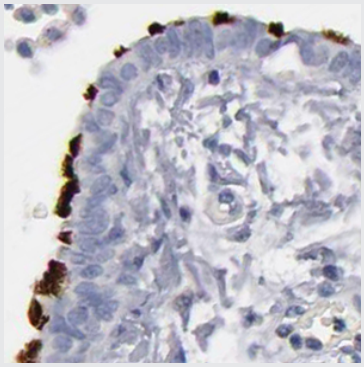
The established golden standard for visualizing proteins at a subcellular level is immunocytochemistry-immunofluorescence (ICC-IF).

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.

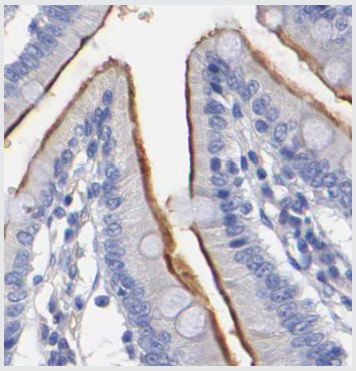
Figure 3 shows that IHC can be used to localize proteins at a subcellular level.



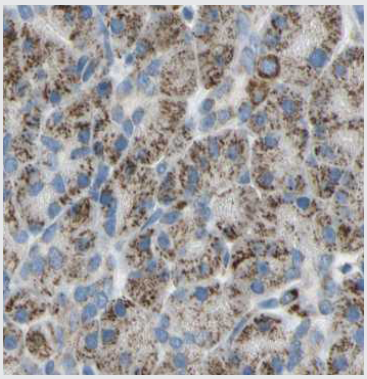
A. Plasma membrane
Anti-CDH1 (HPA004812)
Rectum



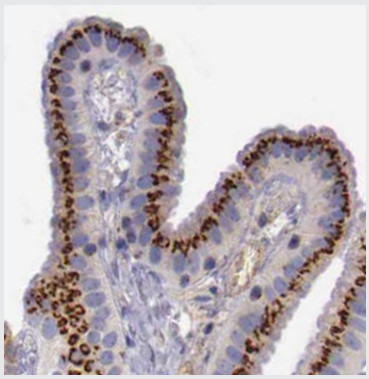
B. Cilia
Anti-ODF2 (HPA001874)
Fallopian tube



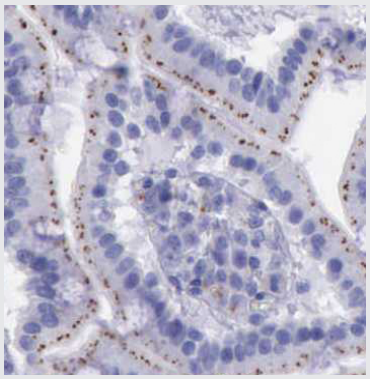
C. Microvilli
Anti-SLC44A2 (HPA003228)
Small intestine



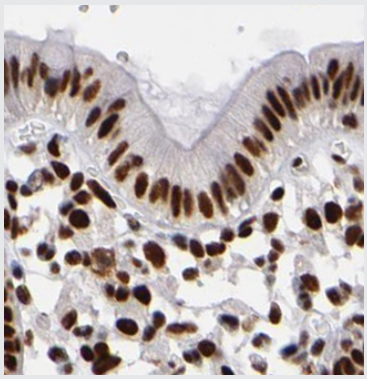
D. Mitochondria
Anti-ACADM (HPA006198)
Pancreas



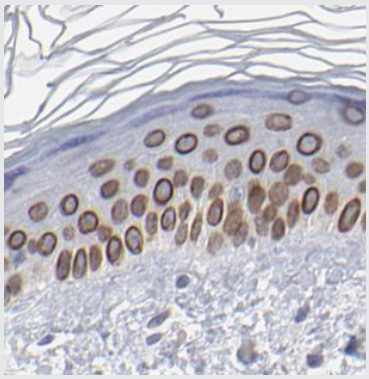
E. Golgi
Anti-GOLGA5 (HPA000992)
Gall bladder



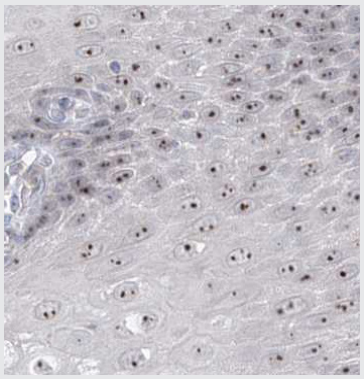
F. Lysosomes
Anti-GLA (HPA000966)
Duodenum



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. (**A-C**) IHC for recognition of cell membrane-related proteins. (**D-F**) IHC on proteins expressed in different cytoplasmic compartments. (**G-I**) IHC on proteins expressed in different nuclear structures. Recognition of target antigen is represented by brown color.

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.

ABOUT ATLAS ANTIBODIES

Atlas Antibodies is a Swedish biotechnology company that facilitates leading research worldwide through manufacturing and providing primary antibodies and protein standards for targeted proteomics using mass spectrometry.

VERY RELIABLE ANTIBODIES

Atlas Antibodies is the original manufacturer of over 21,000 primary antibodies targeting the majority of human proteins. Building on our heritage with the Human Protein Atlas project, we provide highly validated reagents that enable leading research in biology, diagnostics, and medicine. All our products are rigorously evaluated for specificity, reproducibility and performance and characterized in multiple applications. Our team of researchers develops the next generation of innovative and reliable tools, fundamental to advancing research in neuroscience, oncology, cell biology, stem cells and development.

CREATED BY THE HUMAN PROTEIN ATLAS

With our roots in the Human Protein Atlas project, an integration of antibody-based imaging, proteomics, and transcriptomics, our antibodies are affinity-purified, reproducible, selective, and specific for their target proteins through our enhanced validation process. Our Triple A Polyclonals™ are developed within the Human Protein Atlas project.

VALIDATED BY ENHANCED VALIDATION

We take great care to validate our antibodies in IHC, WB, and ICC-IF. Our antibodies are validated in all major human tissues and organs and 20 cancer tissues. Each antibody is supported by over 500 staining images. As an additional layer of security, we perform Enhanced Validation. By using 5 different enhanced validation methods we validate our antibodies for each combination of protein, sample, and application. Discover our Triple A Polyclonals™ and PrecisA Monoclonals™ antibodies targeting the majority of human proteins in cells, tissues, and organs.

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Made by researchers for researchers our products are used all over the world and referenced in 1000s of scientific peer-reviewed papers.

WE SUPPORT YOUR RESEARCH

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Atlas Antibodies Advanced Polyclonals.
Triple A Polyclonals™ are rabbit polyclonal primary antibodies developed within the Human Protein Atlas project. IHC characterization data from 44 normal and 20 cancer tissues is available on the Human Protein Atlas portal. Available as **25 µL** and **100 µL** unit size.

Precise. Accurate.Targeted.
PrecisA Monoclonals™ are mouse monoclonal primary antibodies developed against a number of carefully selected targets. Clones are selected to recognize only unique non-overlapping epitopes and isotypes. Available as **25 µL** and **100 µL** unit size.

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