ATLAS ANTIBODIES

Immunohistochemistry in Human Tissues using Triple A Polyclonals

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. Each year protein expression and localization data of approximately 2,000 new proteins are added to the portal. In April 2013, Triple A Polyclonals have been used to analyze protein expression of more than 13,000 human genes, corresponding to 65% of the proteome. By the end of 2015, a first draft of the localization of the full human proteome will be available.

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 atlas for tissue expression and localization is built up for each protein with an available specific antibody. TMAs used within the Human Protein Atlas project include samples from 48 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].

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 (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.

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 and Western Blots.

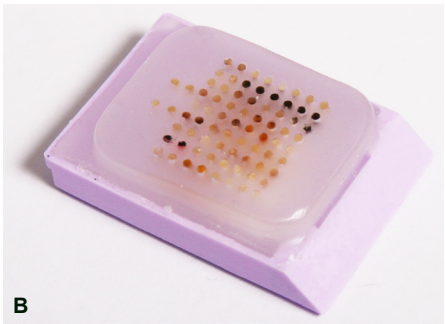


Figure 1.
Cylinders from donor blocks are extracted and inserted into a recipient block. **A**) Donor blocks of formalin fixed, paraffin embedded human tissues. **B**) Recipient block (Tissue Microarray) representing 48 different human normal tissue types ready to be sectioned and used for IHC analysis.

AAA POLYCLONALS
Designed by researchers, for researchers.

THE HUMAN PROTEIN ATLAS

The Human Protein Atlas is a public web portal managed by an academic project that aims to map the human proteome in a period of 10 years. More than 700 IHC, WB and IF images are presented for each antibody against human targets.

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.

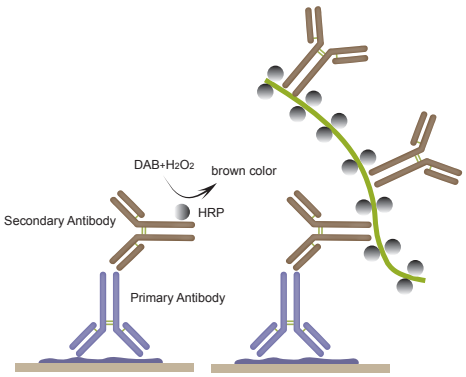


Figure 2.
Schematic figure of the immunohistochemical staining reaction. Triple A Polyclonals are used as primary antibodies and the secondary antibody is labeled with the enzyme HRP. HRP forms a complex with the substrate H_2O_2 and in the presence of the chromogen DAB, a brown color can be visualized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

Scanning and Annotation

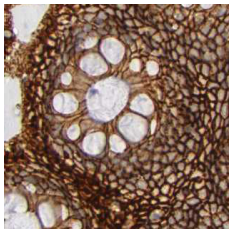
All immunostained slides are scanned to generate high-resolution images. More than 7 terabyte image data is generated each month. The images representing immunostained tissue sections (TMAs) are analyzed and annotated by certified pathologists using a web based annotation software. All images and annotations are published and freely available at the Human Protein Atlas portal (proteinatlas.org).

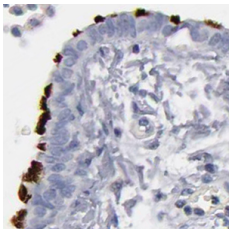
Subcellular analysis using IHC

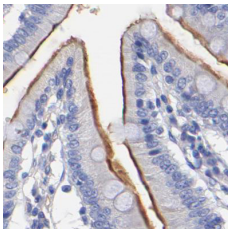
Immunofluorescence-based imaging, with its advantage of high resolution and sensitivity, remains an established golden standard for visualization of proteins at a subcellular level. In addition, immunofluorescence allows the use of several different antibodies tagged with different fluorophores simultaneously, which enables for a more detailed analysis of subcellular localization patterns.

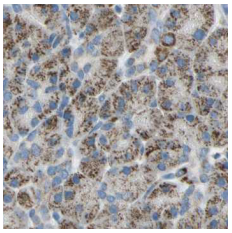
Data on where proteins are localized within a cell provides important information as to what basic functions a protein may have as well as a possibility to map possible other interacting proteins.

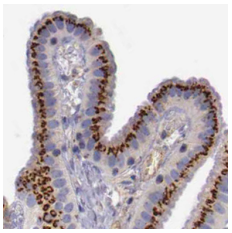
A vast majority of studies based on immunofluorescence are performed on cultured cells with the disadvantage of not being able to analyze cells in their natural tissue context. In addition to the subcellular protein profiling available through immunofluorescence, localization information at a subcellular level can be achieved using Triple A Polyclonals also in immunohistochemistry (Figure 3). Figure 3 A-C show examples of immunohistochemical stainings using Triple A Polyclonals 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.

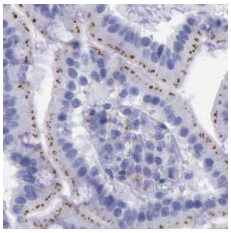

Plasma Membrane, Anti-CDH1 (HPA004812), rectum

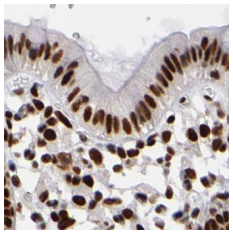

Cilia, Anti-ODF2 (HPA001874), fallopian tube

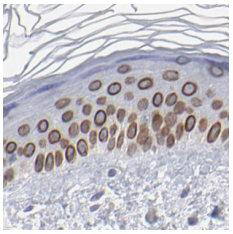

Microvilli, Anti-SLC44A2 (HPA003228), small intestine


Mitochondria, Anti-ACADM (HPA006198), pancreas


Golgi, Anti-GOLGA5 (HPA000992), gall bladder


Lysosomes, Anti-GLA (HPA000966), duodenum


Nucleus, Anti-MRE11A (HPA002691), colon


Nuclear membrane, Anti-SYNE2 (HPA003435), skin

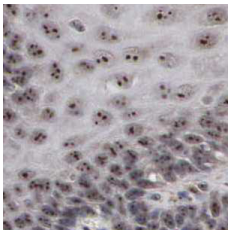

Nucleoli, Anti-A4GALT (HPA001141), esophagus

Figure 3.
IHC stainings showing subcellular localization using Triple A Polyclonals. 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 48 different human normal tissue types and 20 different types of cancer are used for protein localization analysis.
- For each Triple A Polyclonal, more than 500 IHC images are publicly available on the Human Protein Atlas web portal proteinatlas.org.
- Each month the Human Protein Atlas project produces 30 TMAs, performs IHC-analysis on 600 new antibodies and manually annotates the resulting IHC images from 240 antibodies.
- By the use of Triple A Polyclonals 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.

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