ATLAS ANTIBODIES

Development and Validation of Triple A Polyclonals

Triple A Polyclonals

Triple A Polyclonals are highly characterized antibodies with all characterization data for each target protein publically accessible through the Human Protein Atlas (HPA) portal (proteinatlas.org). The uniqueness and low cross reactivity of the Triple A Polyclonals originate from a thorough selection of antigen regions, affinity purification of the polyclonal antibodies, validation using several methods and a stringent selection of approved antibodies.

Antibody Development

The Triple A Polyclonals are developed and validated within the Human Protein Atlas project[1,2,3]. The project was established to allow for a systematic genome-based exploration of the human proteome using antibodies. This is accomplished by combining high-throughput generation of Triple A Polyclonals with protein profiling in a multitude of human tissues and cells. April 2013, 15,100 human proteins have been characterized on the HPA portal, here exemplified by the cell surface antigen CD44 (Figure 1), the RNA binding protein FUS (Figure 2), the intermediate filament protein Nestin (Figure 3) and the transcription factor OLIG2 (Figure 4). Each year protein expression and localization data of approximately 2,000 new proteins are added to the portal. By 2015, a first draft of the localization of the full human proteome will be ready.

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.

Antigen Selection

The Triple A Polyclonals are developed against recombinant human Protein Epitope Signature Tags (PrESTs) of approximately 50 to 150 amino acids[4]. These protein fragments are designed, using a proprietary software, to contain unique epitopes present in the native protein suitable for triggering the generation of antibodies of high specificity. This is achieved by a complete human genome scanning to ensure that PrESTs with the lowest homology to other human proteins are used as antigens. In addition, signal peptides and transmembrane regions are avoided.

Affinity Purification

Purified Triple A Polyclonals are generated by stringent affinity purification using the PrEST antigens as affinity ligands. The purification is performed using a three-step immunoaffinity-based purification protocol, including a tag (HisABP)-specific depletion step, a PrEST-specific capture and finally a buffer exchange by size exclusion chromatography to obtain an optimal environment for long-term antibody storage[5].

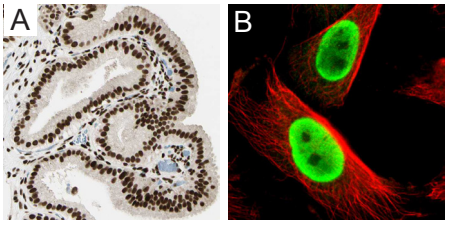

Figure 2.
FUS binds DNA and is suggested to play a role in maintenance of genomic integrity. A) IHC staining of gall bladder tissue showing nuclear positivity in glandular cells. B) IF staining of cell line U-251MG shows positivity in nuclei, but not nucleoli. The stainings are achieved using the Anti-FUS antibody HPA008784.

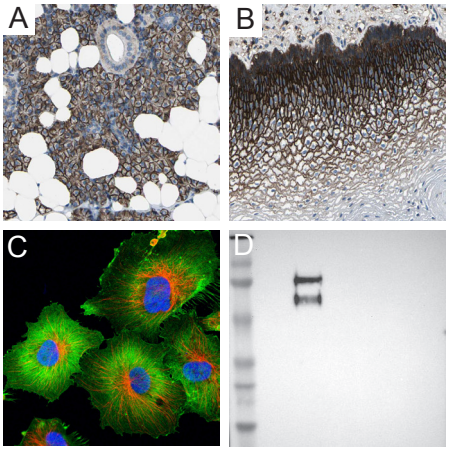

Figure 1.
CD44 is a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration. A) IHC staining of salivery gland tissue showing membranous positivity in glandular cells. B) IHC staining of esophagus tissue showing cytoplasmic and membranous positivity in squamous epithelial cells. C) IF staining of cell line U-251 MG shows positivity in plasma membrane. D) WB showing band of expected size (target weight: 82, 79, 77, 74, 27 kDa. Lane 1: Marker [kDa]: 220,112,84,47,32,26,16.8 ; Lane 2: RT-4; Lane 3: U-251MG sp; Lane 4: Plasma; Lane 5: Liver; Lane 6: Tonsil. The stainings are achieved using the Anti-CD44 antibody HPA005785.

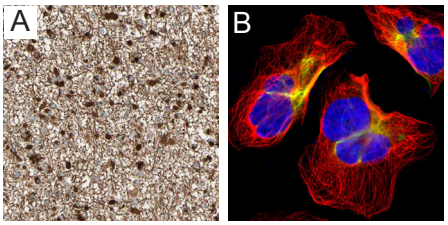

Figure 3
Nestin may play a role in the trafficking and distribution of intermediate filament proteins and potentially other cellular factors to daughter cells during progenitor cell division. A) IHC staining of malignant glioma shows positive staining of tumor cells and nerve fibers. B) IF staining of cell line U2-OS shows positivity in cytoskeleton. The stainings are achieved using the Anti-NES antibody HPA007007.

ATLAS ANTIBODIES

Antibody Validation

Protein Array (PA)

The specificity and purity of the generated antibodies are initially validated by PAs[5] in which a large set of human recombinant protein fragments (PrESTs) is spotted on a microarray and the antibody specificity is determined using a fluorescent-based analysis.

Immunohistochemistry (IHC)

IHC validation is performed for target detection and localization on tissue, cell and subcellular level[6]. Protein expression data is obtained from 48 normal human tissue samples in triplicates, 432 human cancer samples covering the 20 most common cancer types and up to 12 patients for each cancer type. In addition, 59 cells and cell lines are immunochemically stained.

Western Blot (WB)

The antibodies are characterized by WB for target detection and size validation in tissue extracts from liver and tonsil, pooled human plasma depleted of IgG and albumin and cell extracts from two human cell lines. In addition, a selection of antibodies is tested in over-expressed lysates (Origene Technologies).

Immunofluorescence (IF)

Three human cell lines are analyzed for each antibody for more detailed subcellular localization information using confocal microscopy and immunofluorescence[7,8,9]. Successful ongoing efforts are performed in the same cell lines showing evidence of transcript presence, strengthening the antibody validation.

Annotation

Immunohistochemical images of normal and cancer tissues and cells are examined and annotated by certified pathologists. The obtained data from the IHC, WB and IF analyses are compared to known literature and bioinformatics data for each target protein.

Approval of Triple A Polyclonals

The main objective of the HPA project has been to generate antibodies against each human protein and to use these to explore the human proteome. This is done in a highly iterative workflow with rigid quality control in several steps (proteinatlas.com).

The approval of the Triple A Polyclonals relies on a combined validation of the experimental results from IHC, WB and IF and information obtained via bioinformatics prediction methods and literature (for example presence of signal peptide, transmembrane regions or other localization signals). When literature is inconclusive, or when the protein target is expressed in tissues not included in the microarray setup (such as developmental tissues), validation of antibodies is difficult. An important objective of the HPA project has therefore been to generate paired antibodies with non-overlapping epitopes towards the same protein target, allowing the results and validation of one antibody to be used to validate the other. It is reassuring that for the majority of the cases where two separate antibodies exist to the same protein target, the IHC analysis gives identical or similar staining patterns. Observed discordant IHC patterns may be explained by the presence of protein isoforms, such as splice variants or post-translationally modified proteins.

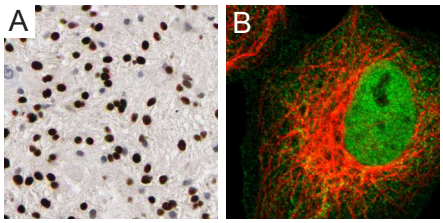

Figure 4.
OLIG2 is a transcription factor required for oligodendrocyte and motor neuron specification in the spinal cord. A) IHC staining of malignant glioma shows nuclear positivity in tumor cells. B) IF staining of cell line U2-OS shows positivity in nucleus, plasma membrane and cytoplasm. The stainings are achieved using the Anti-OLIG2 antibody HPA003254.

References:

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

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

3) Asplund A et al. Antibodies for profiling the human proteome - The Human Protein Atlas as a resource for cancer research. Proteomics. 2012 Jul;12(13):2067-77.

4) Berglund L et al. A whole-genome bioinformatics approach to selection of antigens for systematic antibody generation. Proteomics. 2008 8(14):2832-9.

5) Nilsson P et al. Towards a human proteome atlas: High-throughput generation of mono-specific antibodies for tissue profiling. Proteomics. 2005 5(17):4327-37.

6) Pontén F, Jirström K, Uhlén M. The Human Protein Atlas - a tool for pathology. J Pathology. 2008 216(4):387-93.

7) Lundberg E et al. Creation of an antibody-based subcellular protein atlas. Proteomics. 2010 10(22):3984-96.

8) Lundberg E et al. Defining the transcriptome and proteome in three functionally different human cell lines. Mol Syst Biol. 2010 6:450.

9) Stadler C et al. Immunofluorescence and fluorescent-protein tagging show high correlation for protein localization in mammalian cells. Nat Methods. 2013 Apr;10(4):315-23.

Summary

• High specificity of Triple A Polyclonals is gained through thorough selection of unique antigen regions based on sequence similarity searches against all human proteins.

• Triple A Polyclonals are tested in a series of validation steps; protein array, WB, IHC, IF and literature comparison.

• Approval and subsequent release on the Human Protein Atlas is based on consistency with literature, bioinformatics data and similariry to other antibodies against the same target.

• All characterization data (IHC, IF and WB images) is publicly available on the Human Protein Atlas portal (proteinatlas.org).

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