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. In October 2015, more than 17,000 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). The Human Protein Atlas is being continuously updated with 1-2 releases each year; including new features and updated protein and RNA expression information for additional human genes.

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].

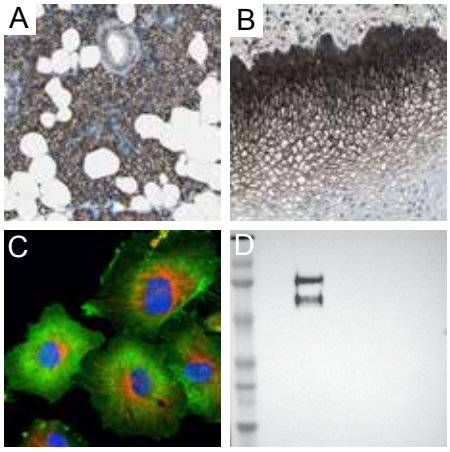

Figure 1.
CD44 is a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration. A) IHC staining of salivary gland tissue showing membranous positivity in glandular cells. B) IHC staining of esophagus tissue showing cytoplasmic and membranous positivity in squamous epithelial cells. C) ICC-IF staining of cell line U-251 MG shows positivity in plasma membrane. D) WB showing band of expected size in cell line U-251 MG (Marker [kDa]: 220, 112, 84, 47, 32, 26, 17). The other tested lysates were negative. The stainings are achieved using the Anti-CD44 antibody HPA005785.

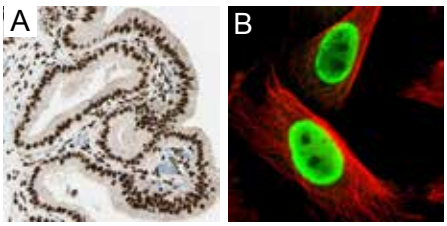

Figure 2.
FUS binds DNA and is suggested to play a role in maintenance of genomic integrity. A) IHC staining of gall bladder tissue showing nuclear positivity in glandular cells. B) IF staining of cell line U-251MG shows positivity in nuclei, but not nucleoli. The stainings are achieved using the Anti-FUS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

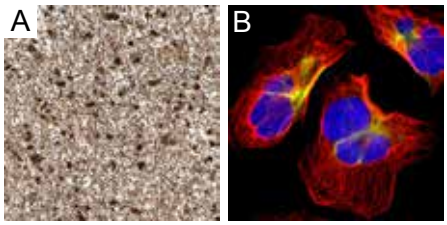

Figure 3
Nestin may play a role in the trafficking and distribution of intermediate filament proteins and potentially other cellular factors to daughter cells during progenitor cell division. A) IHC staining of malignant glioma shows positive staining of tumor cells and nerve fibers. B) IF staining of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

AAA POLYCLONALS
Designed by researchers, for researchers.

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 up to 48 normal human tissue samples in triplicates, 216 human cancer samples in duplicates 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 for a large number of proteins.

Western Blot (WB)

The antibodies are characterized by WB for target detection and size validation in human tissue lysates, plasma, cell extracts from human cell lines and in some cases in over-expressed lysates (Origene Technologies). The validation is continuously expanding in lysates from both human and rodent origin.

Immunocytochemistry-Immunofluorescence (ICC-IF)

A large number of Triple A Polyclonals have been used to achieve detailed subcellular location information of corresponding target proteins. The subcellular analysis has been performed in three selected human cell lines using confocal microscopy and immunofluorescence[7,8,9]. 16 different subcellular locations are being annotated in the IF analysis.

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 ICC-IF analyses are compared to literature, bioinformatics and RNA sequencing 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, RNA sequencing and ICC-IF and information obtained via bioinformatics prediction methods and literature. 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.

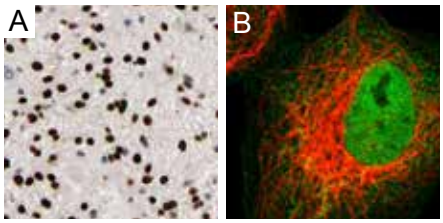

Figure 4.
OLIG2 is a transcription factor required for oligodendrocyte and motor neuron specification in the spinal cord. A) IHC staining of malignant glioma shows nuclear positivity in tumor cells. B) IF staining of cell line U2-OS shows positivity in nucleus and plasma membrane. The stainings are achieved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

References:

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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 and by affinity purification on the PrEST antigen.

• Triple A Polyclonals are tested in a series of validation steps; protein array, WB, IHC and ICC-IF and the obtained results are compared to literature findings and transcript levels of the corresponding target proteins.

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

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

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