Unique Features of Prestige Antibodies®
Powered by Atlas Antibodies

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Prestige Antibodies

Prestige Antibodies are highly characterized antibodies with all characterization data for each target protein accessible through the Human Protein Atlas portal (proteinatlas.org). The uniqueness and low cross-reactivity of the Prestige Antibodies to other proteins are due to a thorough selection of antigen regions, affinity purification of the polyclonal antibodies, validation using several methods, and a stringent selection of approved antibodies.

Prestige Antibody Development

Prestige Antibodies are developed and validated within the Human Protein Atlas project.[1,2] The project was established to allow for a systematic genome-based exploration of the human proteome using antibody-based proteomics. This is accomplished by combining high-throughput generation of Prestige Antibodies with protein profiling in a multitude of human tissues and cells. To date, Prestige Antibodies have been used to analyze protein expression of more than 11,000 human genes, 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 2,500 new proteins are added to the portal. By 2015, a first draft of the localization of the full human proteome will be ready.

Antigen Selection

Prestige Antibodies are developed against recombinant human Protein Epitope Signature Tags (PrESTs) of approximately 50 to 150 amino acids.[3] These protein fragments are designed 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.

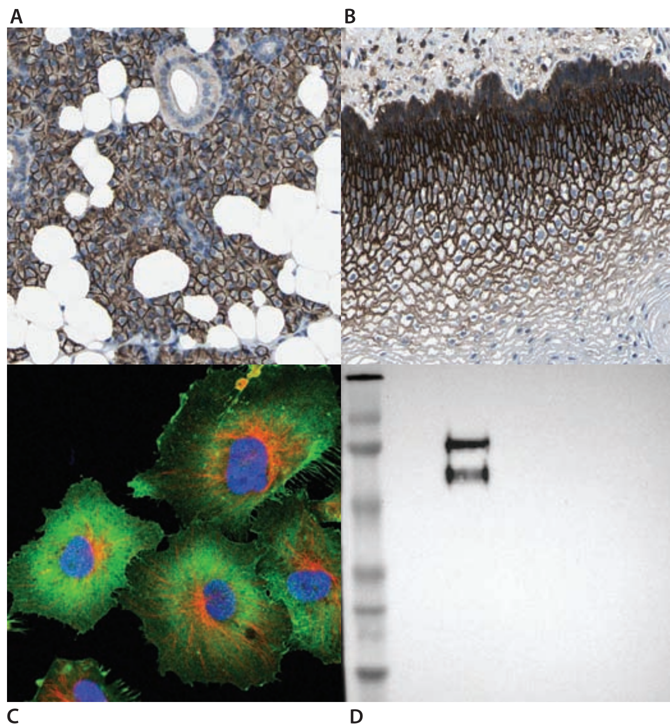

Figure 1: CD44 is a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration. (**A**) IHC staining of salivary gland tissue showing membranous positivity in glandular cells. (**B**) IHC staining of esophagus tissue showing cytoplasmic and membranous positivity in squamous epithelial cells. (**C**) IF staining of cell line U-251 MG shows positivity in plasma membrane. (**D**) WB showing band of expected size (target weight: 82, 81, 79, 78, 77, 74 kDa). Lane 1: Marker [kDa]: 220, 112, 84, 47, 32, 26,16.8; Lane 2: RT-4; Lane 3: U-251 MG sp; Lane 4: Plasma; Lane 5: Liver; Lane 6: Tonsil. **Cat. No. HPA005785**

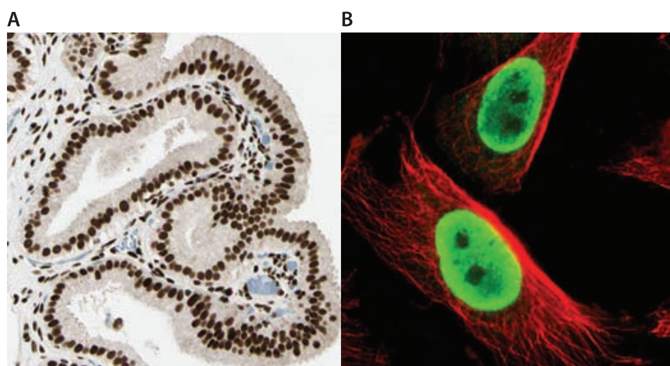

Figure 2: Anti-FUS binds DNA and is suggested to play a role in maintenance of genomic integrity. (**A**) IHC staining of gall bladder tissue showing nuclear positivity in glandular cells. (**B**) IF staining of cell line U-251 MG shows positivity in nuclei, but not nucleoli. **Cat. No. HPA008784**

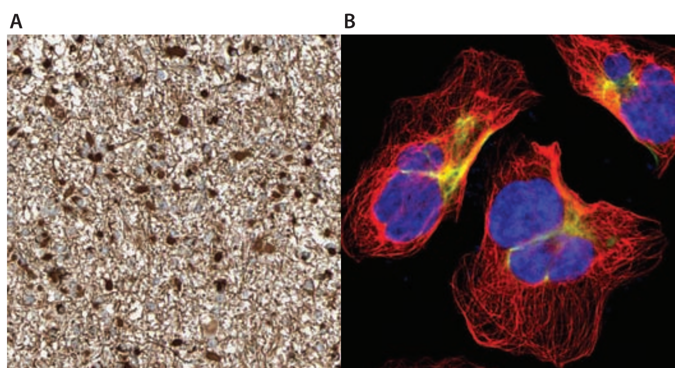

Figure 3: Nestin may play a role in the trafficking and distribution of intermediate filament proteins and potentially other cellular factors to daughter cells during progenitor cell division. (**A**) IHC staining of malignant glioma shows positive staining of tumor cells and nerve fibers. (**B**) IF staining of cell line U-2 OS shows positivity in cytoskeleton. (**Cat. No. HPA007007**)

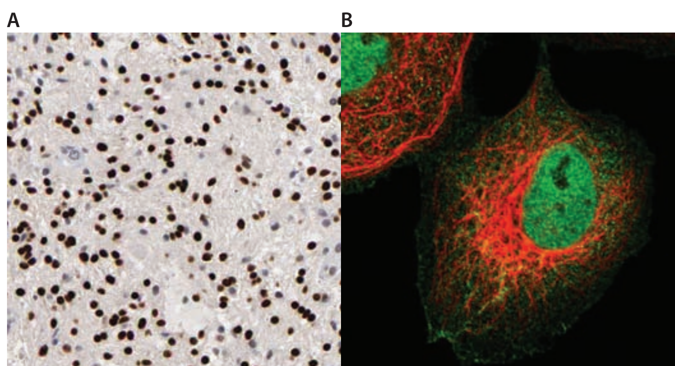

Figure 4: OLIG2 is a transcription factor required for oligodendrocyte and motor neuron specification in the spinal cord. (**A**) IHC staining of malignant glioma shows nuclear positivity in tumor cells. (**B**) IF staining of cell line U-2 OS shows positivity in nucleus, plasma membrane, and cytoplasm. (**Cat. No. HPA003254**)

References:

(1) Uhlén M, et al. A Human Protein Atlas for Normal and Cancer Tissues Based on Antibody Proteomics. *Mol Cell Proteomics* 2005 4(12):1920-1932.

(2) Berglund L, et al. A Genecentric Human Protein Atlas for Expression Profiles Based on Antibodies. *Mol Cell Proteomics* 2008 7:2019-2027.

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

(4) 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.

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

(6) Barbe L, et al. Toward a Confocal Subcellular Atlas of the Human Proteome. *Mol Cell Proteomics* 2008 7(3):499-508.

Affinity Purification

Purified Prestige Antibodies 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.[4]

Prestige Antibody Characterization

Protein Array (PA)

The specificity and purity of the generated antibodies are initially validated by PAs[4] 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 a tissue, cell, and subcellular level.[5] Protein expression data is obtained from 46 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, 56 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 being tested in over-expressed lysates (OriGene Technologies).

Immunofluorescence (IF)

Three human cell lines (U-2 OS, A-431, and U-251 MG) are analyzed for more detailed subcellular localization information using immunofluorescence.[6] 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 is compared to known literature and bioinformatics data for each target protein.

Prestige Antibody Approval

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

The approval of the Prestige Antibodies relies on a combined validation of the experimental results from IHC and WB and information obtained via bioinformatics prediction methods and known 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.

More than half of the HPA antibodies pass the stringent requirements of consistency with literature and/or bioinformatics data, and the resulting Prestige Antibodies are subsequently made public through the Human Protein Atlas.

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. Today, more than 700 IHC, WB, and IF images are presented for each of 14,500 antibodies against human targets, covering 55% of the human proteome.

The antibodies developed and characterized within the Human Protein Atlas project are available to the scientific community as Prestige Antibodies. Prestige Antibodies are co-exclusively distributed by Sigma-Aldrich and Atlas Antibodies in Europe, and exclusively distributed by Sigma-Aldrich outside of Europe.

Summary

- High specificity of Prestige Antibodies is gained through thorough selection of unique antigen regions based on sequence similarity searches against all human proteins.
- Prestige Antibodies are tested in a series of validation steps: protein array, WB, IHC, IF, and literature/bioinformatics comparison.
- For approval and subsequent release on the Human Protein Atlas, stringent requirements of consistency with literature and/or bioinformatics data are needed.
- All characterization data (IHC, IF, and WB images) is publicly available on the Human Protein Atlas (proteinatlas.org).

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