



Prestige Antibodies®

Highly Characterized Antibodies



Sigma's exclusive Prestige Antibodies® powered by Atlas Antibodies could prove priceless to your research!

Prestige Antibodies® powered by Atlas Antibodies

Validated by the Human Protein Atlas (HPA) Project with millions of high-resolution images showing the spatial distribution of proteins in 46 different normal human tissues, 20 different cancer tissue types, and 56 cell lines and primary blood cells.

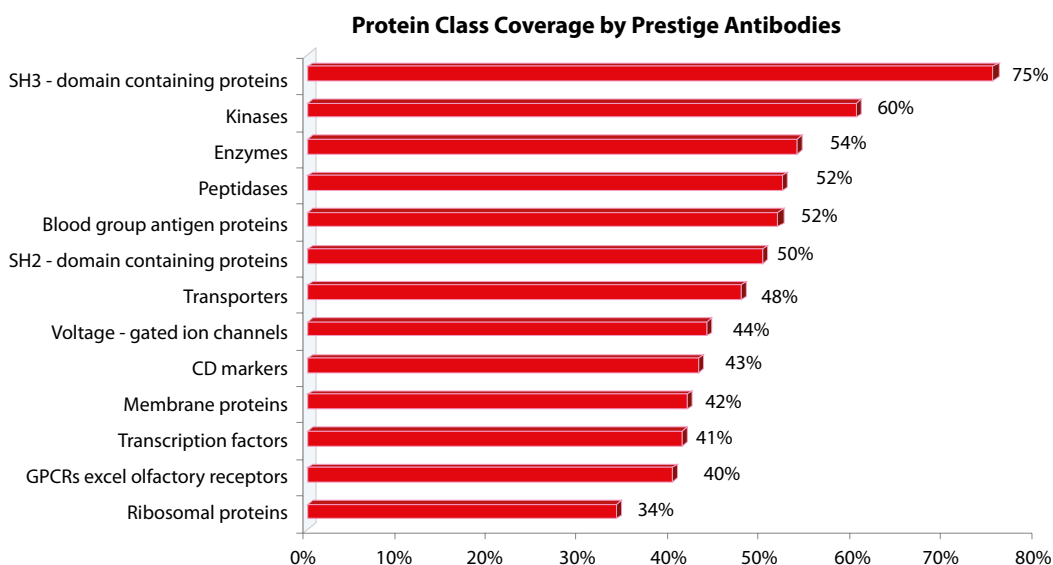
The most highly characterized antibodies in the industry

The Human Protein Atlas Project was established to allow for a systematic 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 assembled in tissue microarrays.

HPA data is released together with application-specific validation performed for each antibody, including immunohistochemistry, Western blot analysis, immunofluorescent based confocal microscopy and protein array assay.

- >10,000 highly validated antibodies available
- 8,300 human protein targets covered
- Developed, characterized, and validated by the Human Protein Atlas Project
- Standardized in universal protocols
- Each antibody is supported by over 700 IHC, IF, and Western blot images
- All data is publicly available on the HPA web site (proteinatlas.org).



Search for Prestige Antibodies at: wherebiobegins.com/prestige

Protein Expression Profiles

Normal Tissues, Immunohistochemistry

Protein expression profiles for each antibody are viewed in triplicate samples in normal tissues. Normal tissues are sampled from 144 different individuals and 138 (46x3) images per antibody are annotated and curated by certified pathologists.

Brown color indicates presence of protein, blue color is unspecific and shows cells and extracellular material.

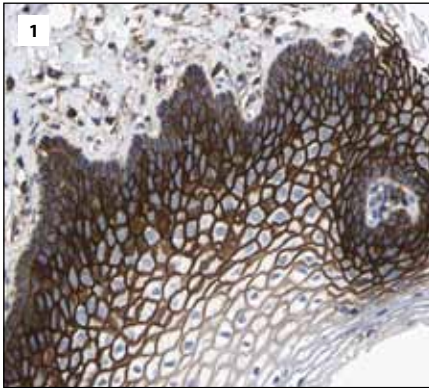


Fig. 1. Vaginal tissue (squamous epithelium) stained with Prestige Antibody anti-CD44 (Cat. No. HPA005785) shows membranous positivity.

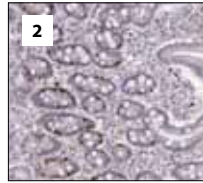


Fig. 2. Duodenum tissue stained with Prestige Antibody anti-MK167 (Cat. No. HPA001164) shows nuclear positivity in a fraction of glandular cells.

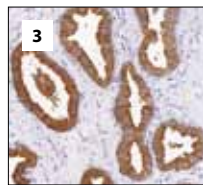


Fig. 3. Prostate tissue stained with Prestige Antibody anti-KLK3 [PSA] (Cat. No. HPA000764) showing cytoplasmic positivity in glandular cells.

Cancer Tissues, Immunohistochemistry

Protein expression profiles for each antibody are viewed in triplicate samples in cancer tissues. Cancer tissues are derived from 216 individuals and 138 (46x3) images per antibody are annotated and curated by certified pathologists.

Brown color indicates presence of protein, blue color is unspecific and shows cells and extracellular material.

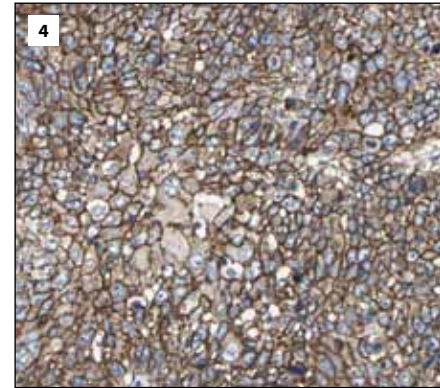
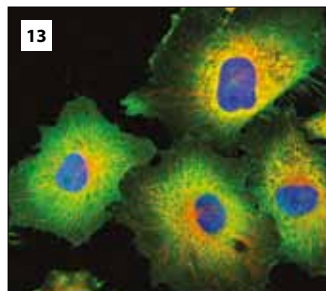
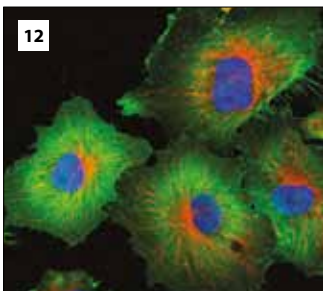
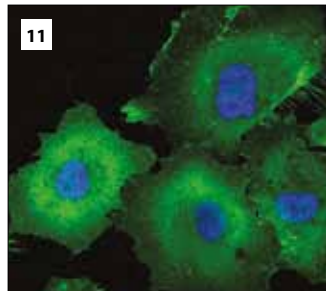
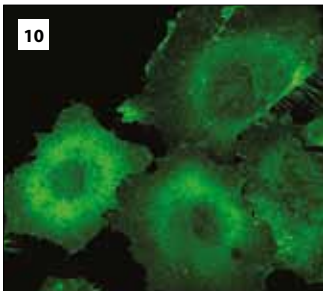


Fig. 4. Cervical cancer tissue (squamous epithelium) stained with Prestige Antibody anti-CD44 (Cat. No. HPA005785) shows membranous positivity.

Immunofluorescent Analysis



A large number of the Prestige Antibodies have been successfully used in Immunofluorescence applications. Confocal microscopy images from three human cell lines A-431, U-2 OS and U-251MG, are available on the Human Protein Atlas.

Staining of the human cell line U-251 MG using the Prestige Antibody anti-CD44 (Cat. No. HPA005785) shows strong positivity of plasma membrane.

The four-color images are acquired in four separate channels and can be viewed separately or in different combinations.

Fig. 10. Staining of plasma membrane in green using anti-CD44 antibody.

Fig. 11. Staining of nuclei (blue) and plasma membrane (green).

Fig. 12. A third channel is turned on for a three-color overlay view of cytoskeleton (red), nuclei (blue) and plasma membrane (green).

Fig. 13. The fourth channel is turned on for a four-color overlay view of endoplasmic reticulum (yellow), cytoskeleton (red), nuclei (blue) and plasma membrane (green).

To view protein expression images, visit the Sigma product detail page, click the link "Human Protein Atlas characterization data".

stry

re viewed in duplicate samples in cancer individuals and the IHC images are annotated

color is unspecific and

Cell Lines, Immunocytochemistry

The HPA displays images of a selection of widely used and well characterized human cell lines, as well as cell samples from normal individuals and leukemia/lymphoma patients. Duplicates from 47 cell lines and 9 samples of primary blood cells result in a total of 118 images per antibody.

Brown color indicates presence of protein, blue color is unspecific and shows cells and extracellular material.

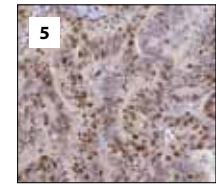


Fig. 5. Colorectal cancer tissue stained with Prestige Antibody anti-MK167 (Cat. No. HPA001164) shows nuclear positivity in glandular cells.

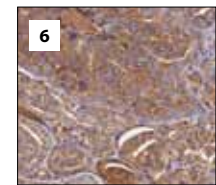


Fig. 6. Prostate cancer tissue stained with protein Cat. No. HPA000764 showing cytoplasmic positivity in glandular cells.

h Prestige Antibody anti-CD44

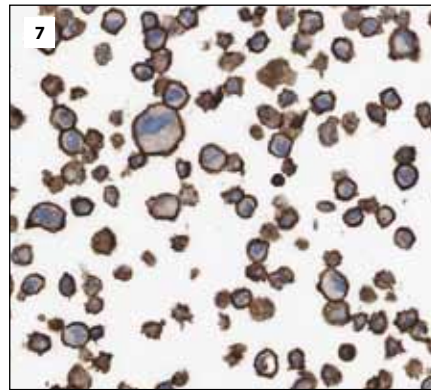


Fig. 7. U-251 MG cells (glioma cell line) stained with Prestige Antibody anti-CD44 (Cat. No. HPA005785) shows membranous positivity.

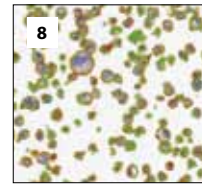


Fig. 8. Area based view representing immunostained areas (%) within the cells. Color code represents a range of immunoreactivity, yellow (weak/moderate), green (moderate/strong), and red (strong).

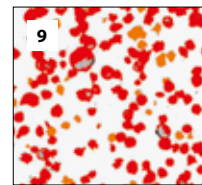


Fig. 9. Object based view representing fraction (%) of immunostained cells. Color code represents range of immunoreactivity, blue (negative/very weak), yellow (weak/moderate), orange (moderate/strong), and red (strong). Negative/very weak areas are transparent.

Western Blot

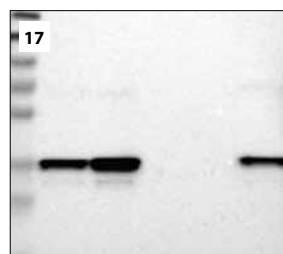
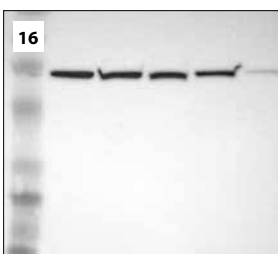
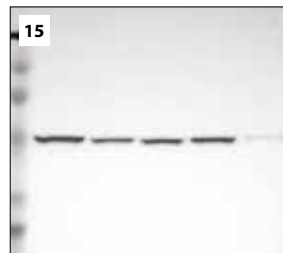
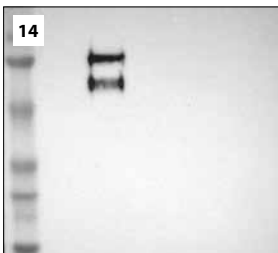
Western blot analysis of total protein extracts are presented for a large number of the Prestige Antibodies. Western blots are performed using a standard sample setup composed of five different human protein lysates: cell-line RT-4, cell line A-431, plasma, liver & tonsil.

Fig. 14. CD44 protein Cat. No. HPA005785

Fig. 15. ATP synthase subunit beta protein Cat. No. HPA001528

Fig. 16. Heat shock 70kDa protein 9 Cat. No. HPA000898

Fig. 17. Annexin A1 protein Cat. No. HPA011272



RNA Expression Analysis

In version 7 of the Human Protein Atlas, the results from RNA expression analysis for each gene have been added in an RNA section. The same three human cell lines used for immunofluorescence analysis, U-2 OS, A-431 and U-251 MG, are being analyzed by RNA-Seq to calculate the RNA levels. RPKM values (number of reads per kilobase gene model and million reads) are used to estimate the expression of each gene in each of the cell lines. The RNA abundance is reported as high, medium, low or not detected. The combined result from the three cell lines is used to classify the gene into five different categories: similar expression, substantially different, slightly different, cell-type specific (only detected in one cell type), and not detected (not detected in any cell line).

RNA Analysis of CD44 Gene

RNA ?>>

Level category Slightly different RNA levels in the three cell lines

Cell line	Description	Origin	RPKM	Abundance level
A-431	epidermoid carcinoma cell line	skin	176.4	high
U-2 OS	osteosarcoma cell line	bone	54.2	high
U-251 MG	glioblastoma cell line	brain	338.6	high

Production of Prestige Antibodies

One major objective of the Human Protein Atlas Project is to produce specific antibodies to human target proteins using a high-throughput production method involving the cloning and protein expression of Protein Epitope Signature Tags (PrESTs). After purification, the antibodies are used to study expression profiles in cells and tissues as well as for functional analysis of the corresponding proteins in a wide range of platforms.

Antibody production starts with the selection of a PrEST. The PrEST fragment is typically 50-150 amino acids in length and is as dissimilar as possible to other proteins. The recombinant PrEST protein is utilized as the antigen to generate affinity purified polyclonal antibodies.

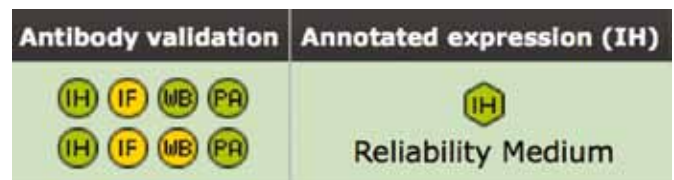
Antibodies are thoroughly tested through a series of quality assurance steps. Plasmid inserts are sequenced to ensure the correct PrEST sequence is cloned. The resulting recombinant protein (including PrEST) is analyzed using mass spectrometry (MS) to assure the correct antigen has been produced and purified.

To assure antigen specificity, antibodies are tested on protein arrays with spotted PrEST fragments. All images generated from IHC staining are annotated and curated by certified pathologists.

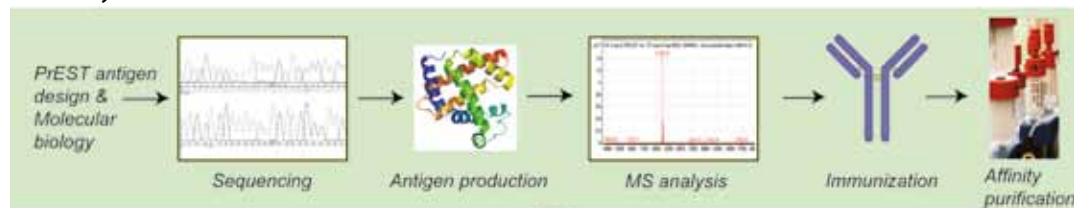
Annotation of Prestige Antibodies

The validation score indicates how well the application data supports the specificity of the antibody towards the expected human target protein. The validation scores are classified into three main categories: Supportive (green), Uncertain (yellow) and Not supportive (red).

In version 7 of the Human Protein Atlas, a new concept of annotated protein expression has been launched. In this effort, several antibodies with non-overlapping epitopes towards the same protein target are used to validate the expression profile of each other. Assembly of data from these antibodies and evaluation of the performance of the antibodies as well as review of available protein/gene characterization data, allows for a knowledge-based interpretation of the protein distribution patterns. A reliability score is generated based on the degree of certainty in the knowledge-based evaluation.



Antibody Production



Multi-functional Characterization



Public web Portal



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