



Prestige Antibodies[®] powered by Atlas Antibodies in Cell and Cell Line Studies

Effective Immunochemical Analysis with Cell Microarrays

Immunohistochemistry on Tissue and Cell Microarrays

Immunohistochemistry (IHC) is a widely used method for determining the level and distribution of protein expression in tissues and cells. With the development of the tissue microarray (TMA) technology, IHC can now be used for the simultaneous analysis of protein expression in a multitude of tissue samples. Within the ongoing Human Protein Atlas (HPA) project (proteinatlas.org)^{1,2,3}, the human proteome is systematically analyzed in 46 normal human tissues and 20 different cancer types using TMAs and automated IHC in a high-throughput setting.

The ability to embed cultured cells in paraffin enabled the construction of cell microarrays (CMAs). As a complement to the TMA, a CMA, including 47 human cell lines and 12 leukemia cell samples, was designed within the HPA project⁴. An objective of this design is to facilitate studies of normal hematopoiesis and differentiation as well as tumor progression. Commonly used cell lines were selected to represent the major forms of solid tumors and hematological malignancies. Cell types not represented in the HPA tissue microarrays were also included. In addition to providing more comprehensive protein expression profiles, CMAs also render many uncontrolled variables equal across the cells, thus improving the possibility for relative protein quantification.

The Human Protein Atlas

Establishment of the HPA project permits 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 were used to analyze protein expression of more than 11,000 human genes. Each year, protein expression and localization data for approximately 2,500 new proteins are added to the portal. By 2015, a first draft of the spatial expression profile of the human proteome will be completed.

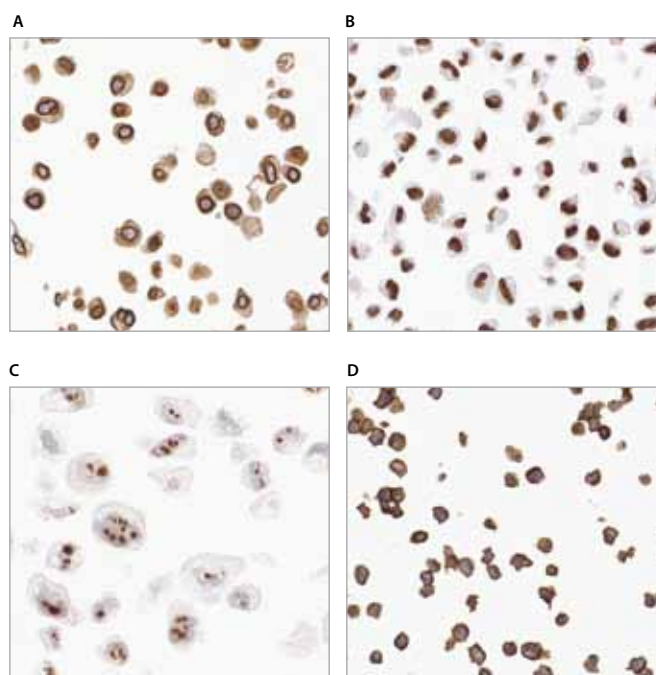


Figure 1. Immunochemical staining showing subcellular localization patterns.

The specific binding of an antibody to its corresponding antigen results in a brown-black staining by a horseradish peroxidase reaction. Blue color of nuclei corresponds to unspecific counter-staining with hematoxylin. **A)** Anti-EMD (HPA000609) stains nuclear membrane in cell line RT-4; **B)** Anti-CSTF2 (HPA000427) stains nucleus in cell line PC-3; **C)** Anti-VSX2 (HPA003436) stains nucleoli in cell line BEWO; and **D)** Anti-NF2 (HPA003097) stains plasma membrane in cell line U-2 OS.



Prestige Antibodies® powered by Atlas Antibodies in Cell and Cell Line Studies
Effective Immunochemical Analysis with Cell Microarrays

Cell Microarrays

Cell microarrays are constructed to allow simultaneous analysis of protein expression in a multitude of cell samples. To enable the assembly of *in vitro* cultured cell lines and primary cells in a microarray format, the cells are first homogeneously distributed in an agarose gel matrix. After polymerization of the agarose, the cell-agarose gel is fixed in 4% formaldehyde and histoprocessed using a vacuum infiltration processor. With this protocol, artificial tissue blocks can be used in a similar manner as paraffin-embedded tissues for cell microarray construction.

The actual CMA is subsequently produced by extracting 0.6 mm cylinders from the cell blocks with a sharp punch and placing them in a recipient block with a core-to-core distance of 1.3 mm in all directions. An automatic TMA arrayer is used for the CMA construction. From one array block, approximately 250 sections can be cut and prepared for immunochemical analysis.

All Prestige Antibodies are analyzed on sections from the CMAs. The resulting protein expression profiles and all immunostaining images showing cellular distribution (**Figure 1**) are shown on the Human Protein Atlas portal (proteinatlas.org).

Image Analysis

The purpose of using CMAs is to gain protein localization information on cellular and subcellular levels, and to achieve relative quantification results. In order to generate non-subjective quantitative data from immunohistochemistry, analysis of immunoreactivity in cells and cell lines in the CMA is performed using automated image analysis⁵.

Immunostained CMA slides are scanned to generate high-resolution digital images. Automated scanning is performed using 40x magnification. Output parameters from the software are always displayed in conjunction with the annotated images and include: number of objects defined as cells in the image; staining intensity (negative, weak, moderate, and strong); and fraction (%) of positive cells. Two overlay images with additional numerical information are presented to facilitate interpretation (**Figure 2**).

Cell Expression Diagram

A cell expression diagram is presented for each Prestige Antibody on the Human Protein Atlas (**Figure 3**). This diagram represents a visualization of normalized protein expression values across the different cells relative to the cell type with the highest level of expression, e.g., PBMC (M36) (**Figure 3**). Cells are grouped according to cellular origin to facilitate functional analysis and identification of proteins with a cell-type-specific expression pattern. Since protein expression correlates with cellular size, the diagram is based on expression levels normalized against the total protein content for each particular cell type (expression values for all analyzed antibodies added together in each cell type)⁵.

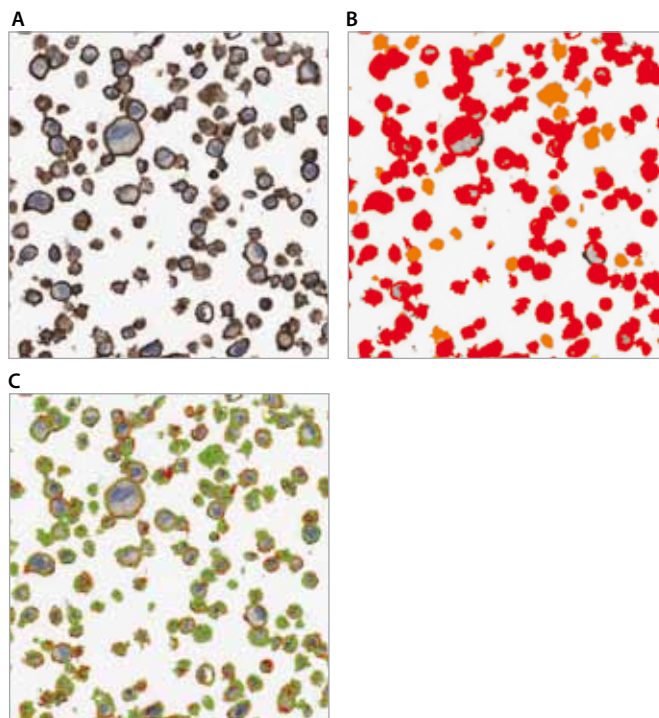


Figure 2. Automated Image Analysis of HPA005785 on the Human Protein Atlas

A) Immunohistochemical staining of Anti-CD44 shows positivity of plasma membrane. **B)** Object-based view representing fraction (%) of immunostained cells. The color code for each cell represents a range of immunoreactivity: blue (negative/very weak), yellow (weak/moderate), orange (moderate/strong), and red (strong) cells. This classification is based on areas of different intensities within each object (cell). **C)** Area-based view representing immunostained areas (%) within cells. The color code represents a range of immunoreactivity: yellow (weak/moderate), green (moderate/strong), and red (strong). Negative/very weak areas are transparent. The annotated intensity score presented on the HPA is deduced from data generated from both the object-based and the area-based analysis.

Prestige Antibodies®

Powered by  **ATLAS**
ANTIBODIES



Prestige Antibodies® powered by Atlas Antibodies in Cell and Cell Line Studies
Effective Immunochemical Analysis with Cell Microarrays

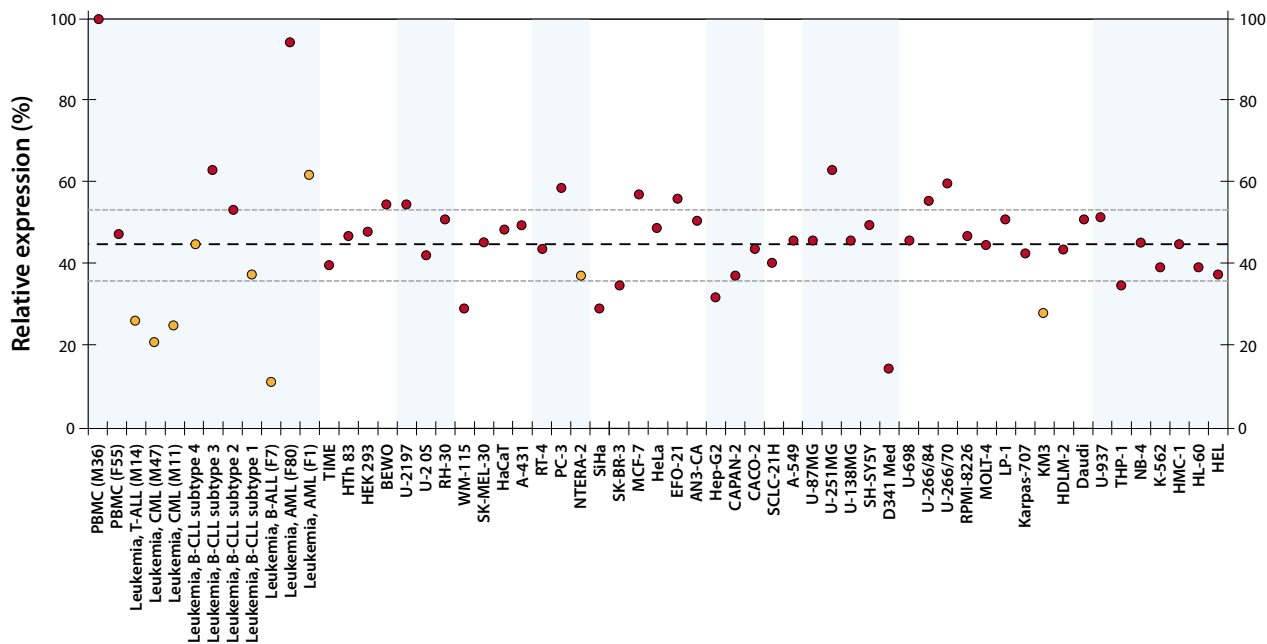


Figure 3. Cell Expression Diagram displayed on the Human Protein Atlas visualizing the expression level for the EMD protein by HPA000609 in the analyzed cell lines and cell samples as estimated using automated image analysis.

Summary

- CMA's enable simultaneous immunochemical analysis of protein expression using Prestige Antibodies in 59 cell lines and primary cell samples.
- Protein localization information on cellular and subcellular levels can be achieved by immunochemical analysis on CMA's using Prestige Antibodies.
- 118 high-resolution immunochemical images from 12 clinical leukemia cell samples and 47 human cell lines are presented for each Prestige Antibody on the Human Protein Atlas portal.

- Automated image analysis objectively scores protein expression.
- Standardized protocols, the use of CMA's, and normalization in reference to total protein expression enable extrapolation of quantitative immunochemical data.

Discover more at wherebiobegins.com/prestige



Enabling Science to Improve the Quality of Life

Order/Customer Service (800) 325-3010 • Fax (800) 325-5052
 Technical Service (800) 325-5832 • sigma-aldrich.com/techservice
 Development/Custom Manufacturing Inquiries **SAFC**® (800) 244-1173
 Safety-related Information sigma-aldrich.com/safetycenter

World Headquarters
 3050 Spruce St.
 St. Louis, MO 63103
 (314) 771-5765
sigma-aldrich.com

©2011 Sigma-Aldrich Co. All rights reserved. SIGMA, SAFC, SIGMA-ALDRICH, ALDRICH, FLUKA, and SUPELCO are trademarks belonging to Sigma-Aldrich Co. and its affiliate Sigma-Aldrich Biotechnology, L.P. Sigma brand products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip. Prestige Antibodies is a registered trademark of Sigma-Aldrich Co. and its affiliate Sigma-Aldrich Biotechnology, L.P.