Dissociable Antibody Microarray (DAMA) Staining

Immunochemical staining is a versatile technique in determining both the presence and localization of a protein. This information is of immense value to biomedical research and clinical medicine. Most of the current methods, all of which involve incubating cells with an antibody solution, only allow cell staining with one or a few antibodies at a time. These methods are not suitable for many applications in which the expressions and sub-cellular localizations of a large number of different proteins need to be examined. Although several multiplexed staining methods have been developed, they can only stain a small number of targets at a time.

Dissociable Antibody MicroArray (DAMA™) staining takes advantage of "Dissociable Protein Array" technology, which allows the delivery of a large number of proteins to their targets in a position-addressable manner. In DAMA staining method the antibodies are immobilized on a membrane in such a manner that when they make contact with cells fixed on another support, the antibodies can bind to their respective antigens. When the array support is separated from the cells, the antibodies will be dissociated from the support and remain bound to the antigens. Therefore, the method enables the staining of multiple antibodies simultaneously, each at a pre-determined position.

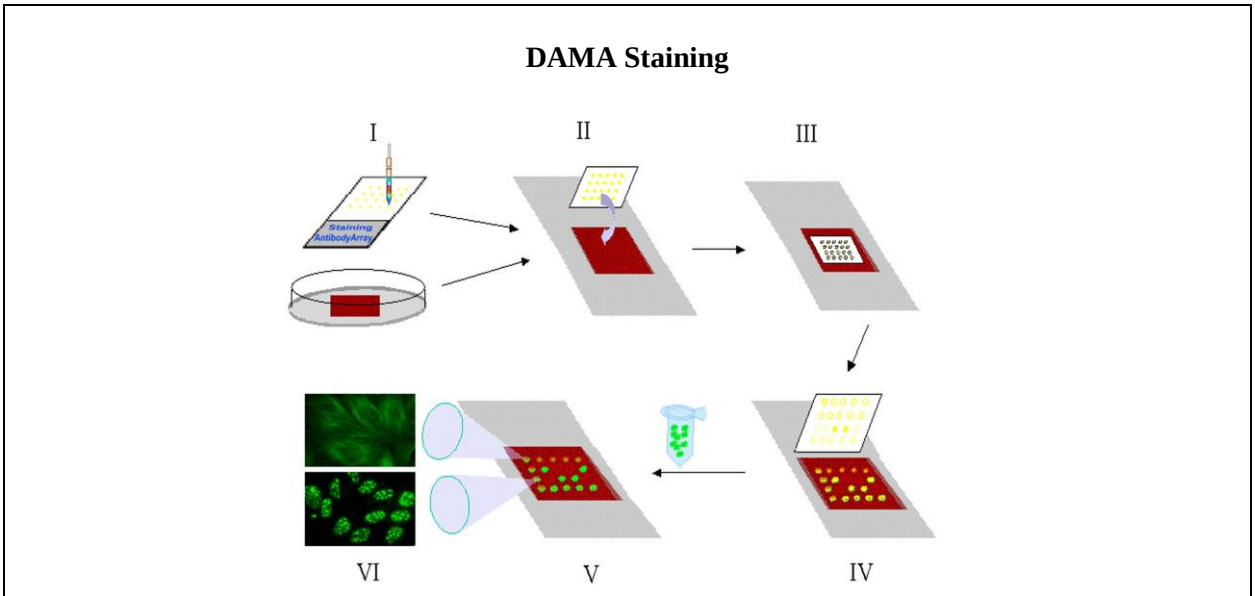

Fig. 1: Overview of DAMA staining technology. **I**, prepare antibody microarray and adherent cells; **II**, make contact between arrays and cells; **III**, Incubate the microarray with the cells to allow antibodies bind their antigens; **IV**, remove array support; **V**, apply secondary antibodies; **VI**, observe the staining under a microscope or a scanner.

DAMA Advantages:

1. High-throughput immunostaining.
2. Without the drawbacks of multiplexing.
3. Easy to implement.
4. Can be combined with other staining methods.
5. Using less antibodies (nanograms) than regular staining.

Applications:

DAMA staining technology offers a high-throughput method for examining *in vivo* protein activities. It has many applications, including:

1. Examining protein expressions
2. Revealing protein sub-cellular localizations

To compare protein expressions between two samples, the staining can be observed via enzyme-mediated color reaction (Fig. 2). Or the staining can be observed under microscope and quantified.

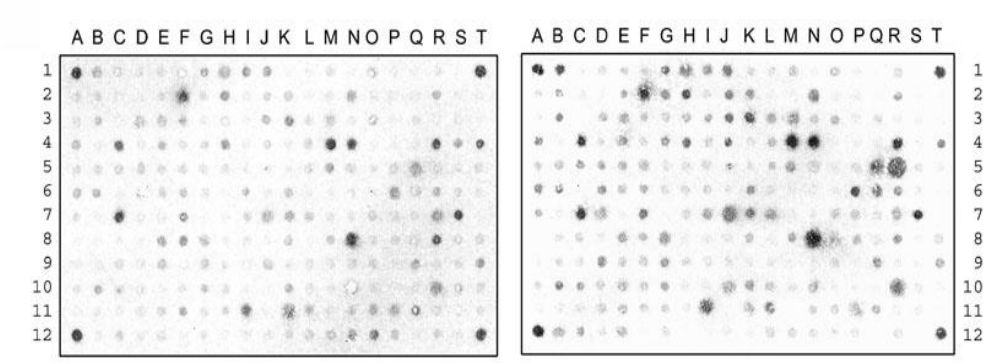

Fig. 2: Comparison of protein expressions between ME180 cells (left) and A431cells (right) using antibody arrays with 240 antibodies. Alkaline phosphatase-labeled secondary antibodies were used and the staining was visualized by color reaction with BCIP/NBT as substrates

Knowledge of a protein's sub-cellular localization can provide important information about the protein's functional state. For example, change of a transcriptional factor's location from cytoplasm to nucleus often suggests its activation. When observed under fluorescent microscope, AntibodyArray™ staining can reveal sub-cellular localizations of many proteins simultaneously.

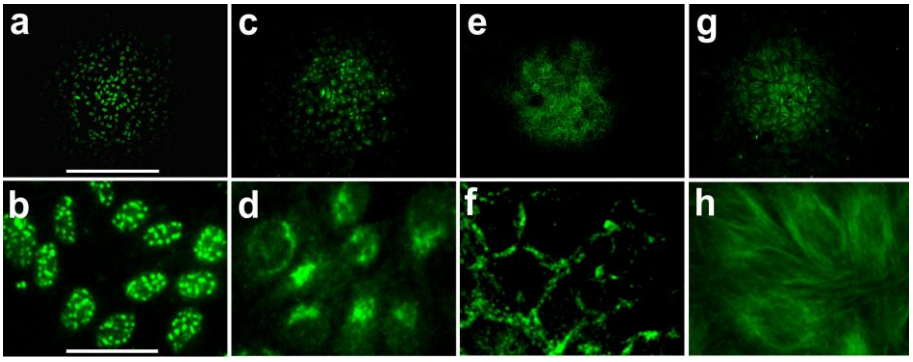

Fig. 3: Fluorescent staining with staining AntibodyArray™. An array of 200 rabbit polyclonal antibodies were used and the staining at four positions are shown here as representatives. **a, b**, transcriptional factor IRF1. **c, d**, signaling molecule 14-3-3 β. **e, f**, cell adhesion protein β-catenin. **g, h**, transcriptional factor Ets-1. Low magnification (**a**, **c**, **e**, **g**, and **i**) shows the stained cells and surrounding non-stained area; and high magnification shows the detailed nuclei localization of IRF1 (**b**), cytoplasmic staining of 14-3-3 β (**d**) and Ets-1 (**h**), and membrane staining of β-catenin at cell-cell contacts (**d**). Scale bar in **a**, 300 μm; Scale bar in **b**, 30 μm.

Simultaneous staining of two proteins (double staining) is a unique tool for studying two functionally related proteins. For example, evidence of protein interactions often includes the demonstration that the proteins co-localize in the same cellular structure. Double staining is also useful when the protein of interest is only expressed in small number of target cells among a heterogeneous cell population, and the protein of interest need to be observed together with a protein marker which is used to denote the target cells. Array staining is unique in that it allows the examination of multiple proteins individually as well as simultaneously in the same cell preparation.

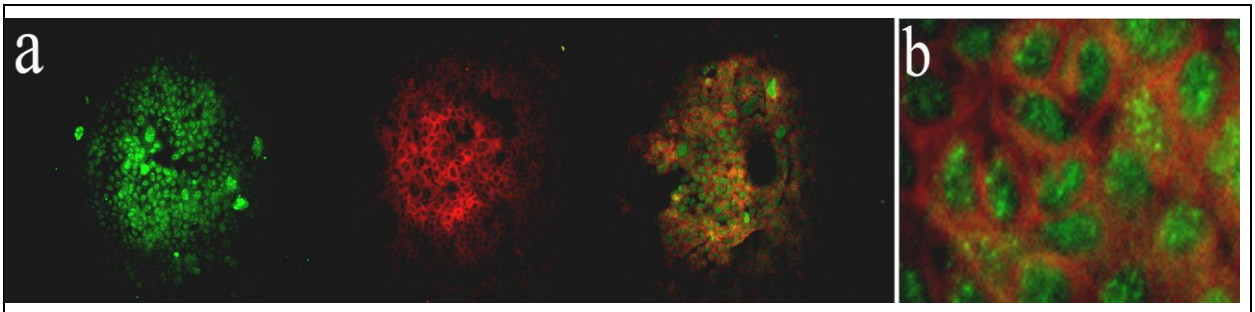

Fig.4: Fluorescent double staining withAntibodyArray™.**a**, Low magnification of A431 cells staining with an array containing rabbit anti-YY1 antibodies (left in green), mouse anti-p130^{cas}antibodies (middle in red), and both YY1 (right in green) and p130^{cas} (right in red) antibodies at neighboring positions. Goat anti-rabbit Cy2-labeled secondary antibodies and goat anti-mouse Cy3-labeled secondary antibodies were used. **b**, Enlarged view of the double staining of YY1 (green) and p130^{cas}(red) from **a**.

Hypromatrix's Staining Antibody Microrrays are designed for the following applications:

1. Revealing a protein's novel functions;
2. Understanding molecular mechanisms of protein's function.
3. Dissecting signaling pathways activated by specific stimulations;
4. Screening cellular effects of drug candidates;
5. Discovering novel diagnostic markers.

References:

1. Wang, Y. (2004) Immunostaining with dissociable antibody microarrays. *Proteomics* 4, 20-26.
2. Nature Vol 429, MAY 6, 2004 p101-107 "Proteomics in multiplex"
3. The Scientist: Volume 18 | Issue 18 | 42 | Sep. 27, 2004 "High-Throughput Immunostaining"
4. Song, C. et. al. (*2008).* "Protein Expression Profiling of Breast Cancer Cells by Dissociable Antibody Microarray (DAMA) Staining". *Molecular & Cellular Proteomics 7:163-169.*
5. Fu G, et. al. (2010). Protein Subcellular Localization Profiling of Breast Cancer Cells by Dissociable Antibody MicroArray (DAMA) Staining. *Proteomics.*10(8):1536-44.

For additional information regarding other applications and custom-tailored staining arrays, please contact Hypromatrix, Inc.