

NEW: Screen Protein Domains for Histone Binding Partners

Active Motif was the first company to introduce an array capable of analyzing antibody and protein interactions with histone post-translational modifications (PTMs). Our unique MODified™ Histone Peptide array offers the ability to screen 384 different modification combinations to evaluate cross-reactivity between individual modifications as well as investigating the effects of adjacent modifications on binding affinity. Active Motif has expanded our product offering with our new MODified™ Protein Domain Binding Kit. This assay is designed for researchers interested in screening their protein domains (*e.g.* bromodomains, chromodomains, *etc.*) for reactivity with specific histone PTMs.

What are protein domains?

The epigenetic information that exists in the form of post-translational modifications on histone tails is generated, interpreted and edited by proteins that are coined “writers”, “readers” and “erasers”. There are several classes of protein domains that influence gene regulation and chromatin remodeling by interacting with specific histone PTMs. See [Table 1](#) for a list of some common chromatin remodeling protein domains.

Our current understanding of these protein domains has been limited by the lack of appropriate tools to fully interrogate their binding specificity. Active Motif’s MODified™ Histone Peptide Array provides a solution to this problem as it enables researchers to screen a large panel of histone modifications in a single experiment. The results provide informa-

tion on protein domain interactions with specific histone modifications, and also reveals the influence that adjacent modifications may play on protein binding.

How does it work?

Active Motif’s MODified Histone Peptide Array contains modifications to study histone acetylation, methylation, phosphorylation or citrullination on histones H2A, H2B, H3 and H4. Each peptide is spotted in duplicate on the array to analyze the reproducibility of the binding events.

The MODified Protein Domain Binding Kit was designed to be used in conjunction with the peptide arrays. The simple assay works like a Western blot. The array is first incubated in blocking buffer. Then, the His-tagged protein domain of interest is added to the array and

detected via an anti-His tag antibody and an HRP-conjugated secondary antibody. ECL detection is used to produce a signal. An image of the array is captured using a luminescent imaging system.

Free Array Analyze Software

Active Motif offers free Array Analyze software that can be downloaded from our website to analyze the intensity of the spots. Information about binding partners can be interrogated through the software program to help identify the histone modifications associated with binding of the protein domain ([Figure 1](#)). We have recently updated our Array Analyze software package to include new features that make the software easier to use and simplify the way the results are displayed. Download a copy of our new software program today at www.activemotif.com/modified.

Domain	Binding Site	Function	Examples
Bromo domain	Acetylated lysine residues on histones or other proteins	Regulates chromatin structure and gene expression as part of histone acetyltransferases or chromatin remodeling factors	TAF _{II} 250, PCAF, GCN5
Chromo domain	Methylated lysine residues on Histone H3	Associated with the assembly of protein complexes on chromatin	HPIβ, MPP8, CHDI
Tudor domain	Methylated lysine or arginine residues on Histone H3 and H4	It may be involved in RNA binding, DNA damage response and chromatin modification	JMJD2A, 53BP1, SMN
MBT domain	Methylated lysine residues on Histone H3 and H4	The exact function is unknown, but MBT often appears as repeats in proteins associated with transcriptional repression	L(3)MBTL, CGI-72

Table 1: Examples of some common protein domain classes and their associated binding specificity and function.

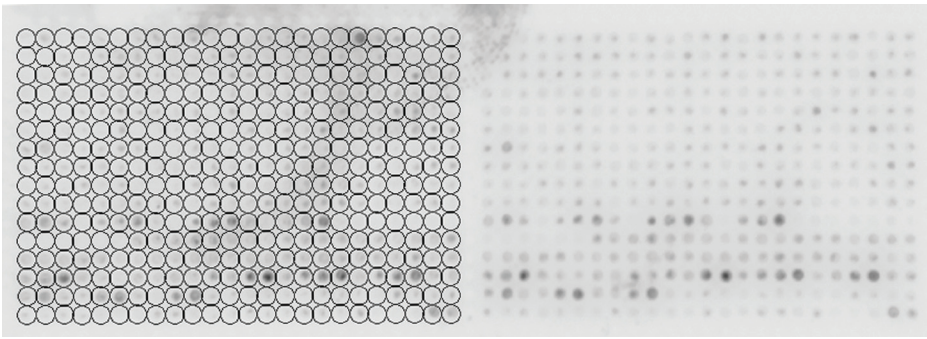
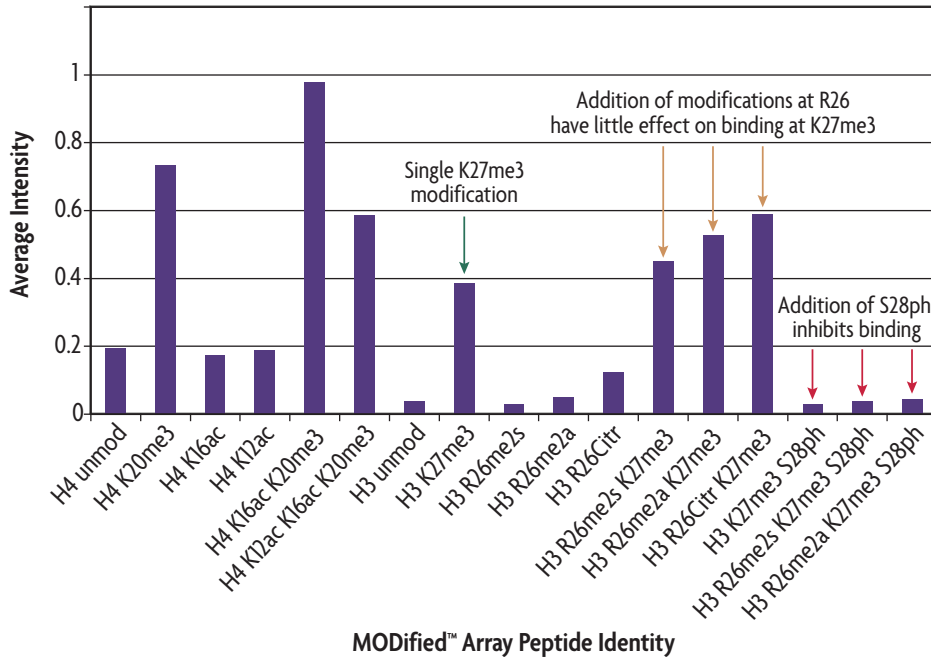


Figure 1: Analysis of the JMJD2A protein domain using the MODified Protein Domain Binding Kit and the MODified Histone Peptide Array. The His-tagged Tudor domain protein, JMJD2A, was used at a 50 nM concentration with the MODified Protein Domain Binding Kit and the MODified Histone Peptide Array. An image of the array was captured using a chemiluminescent imaging system.

Binding Specificity of JMJD2A



Top: JMJD2A array image in which the grid overlay on the left half demonstrates the alignment performed by the Array Analyze software program for spot identification.

Bottom: Graphical analysis of selected data from the Array Analyze software program which was used to calculate the spot intensity of binding events for the JMJD2A protein domain. The average intensity was calculated for each duplicate on the left and right side of the array and graphed for the peptides listed.

The data shows the specificity of the JMJD2A domain for Histone H4K20me3 and H3K27me3 (green arrow). The presence of additional modifications within the same peptide had varying influences on spot intensity. There was relatively little change when the H3K27me3 modification was present together with modifications at R26 (copper arrows). But, when the S28ph modification was adjacent to K27me3, binding of the JMJD2A protein domain was inhibited greatly (red arrows).

MODified Array Labeling Kit

In addition to the new Protein Domain Binding Kit, the MODified Histone Peptide Arrays can also be used to screen antibodies for cross-reactivity. The MODified™ Array Labeling Kit provides all the reagents needed for the blocking, washing and chemiluminescent detection of the array. HRP-conjugated secondary antibodies for use with either rabbit or mouse primary antibodies, and a positive control antibody that recognizes the c-Myc control spot printed on the peptide array are also included. The c-Myc control antibody can be combined with the antibody you are screening to assist with spot identification within the Array Analyze software.

Active Motif offers a broad selection of recombinant proteins, including Bromodomains, HATs, HDACs, HMTs and HDMs, which can be used to study functional changes and binding specificity on the MODified Histone Peptide Array. To see a full list of available proteins, please visit us at www.activemotif.com/proteins.

Product	Format	Catalog No.
MODified™ Histone Peptide Array	1 array	13001
	5 arrays	13005
MODified™ Protein Domain Binding Kit	5 rxns	13007
MODified™ Array Labeling Kit	5 rxns	13006