

MODified™ Protein Domain Binding Kit

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(version A1)

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Overview

The epigenetic information that exists in the form of histone post-translational modifications (PTMs) 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. Some common chromatin remodeling protein domains include bromodomains, chromodomains and tudor domains to name a few.

The MODified™ Protein Domain Binding Kit is designed to be used in combination with Active Motif’s MODified Histone Peptide Array* to screen His-tagged protein domains for interactions with histones and their post-translational modifications. Each MODified 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. This unique histone array contains up to four separate modifications per 19mer peptide to allow researchers to study not only individual sites, but also the effects of neighboring modifications on recognition and binding.

The MODified™ Protein Domain Binding Kit contains the buffers and reagents needed for analysis of His-tagged protein domain binding specificity towards histone PTMs and chemiluminescent detection of the MODified Histone Peptide Arrays. The MODified Protein Domain Binding Kit contains interaction buffer, wash buffer, anti-His6-tag antibody, HRP-conjugated secondary antibody and ECL reagents for chemiluminescent detection. For added convenience, a positive control histone lysine methyltransferase G9a is included.

Following the interaction of the protein domain with the array and ECL detection, an image is then captured using a luminescent imaging system. Active Motif’s free Array Analyze Software can be used 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.

The MODified Histone Peptide Arrays are available individually, or in packs of five. The arrays are **not** included in the MODified Protein Domain Binding Kit and must be purchased separately.

product	format	catalog no.
MODified™ Histone Peptide Array	1 array	13001
	5 arrays	13005
MODified™ Protein Domain Binding Kit	5 rxns	13007

*CelluSpots™ arrays are manufactured under license by INTAVIS Bioanalytical Instruments AG

Introduction

Epigenetic signals include DNA methylation, non-coding RNA, and post-translational modifications (PTMs) on the N-terminal histone tails. Histone modifications can consist of acetylation, mono-, di- (symmetric or asymmetric in the case of arginine) and tri-methylation of lysine and arginine residues, phosphorylation of serine or threonine residues, citrullination and ubiquitination. These epigenetic marks play a role in the regulation of gene expression and chromatin state¹⁻⁴.

These histone modifications are recognized and bound by specific proteins that are coined 'writers' 'readers' and 'erasers'. Many of these proteins contain highly conserved domains which serve as the building blocks of the protein structure. Some of these domains have a clearly defined function associated with them⁵⁻⁶. A multitude of protein domains exist, such as bromodomains, chromodomains and Tudor domains to name a few. Bromodomains are approximately 110 amino acids folded into a left-handed four α -helical bundle. There are more than 70 identified members of the bromodomain family. Bromodomains are known to bind acetylated lysine residues on histones or other proteins. They regulate chromatin structure and gene expression as part of histone acetyltransferases or chromatin remodeling factors. Please visit www.activemotif.com for a list of bromodomain proteins available from Active Motif.

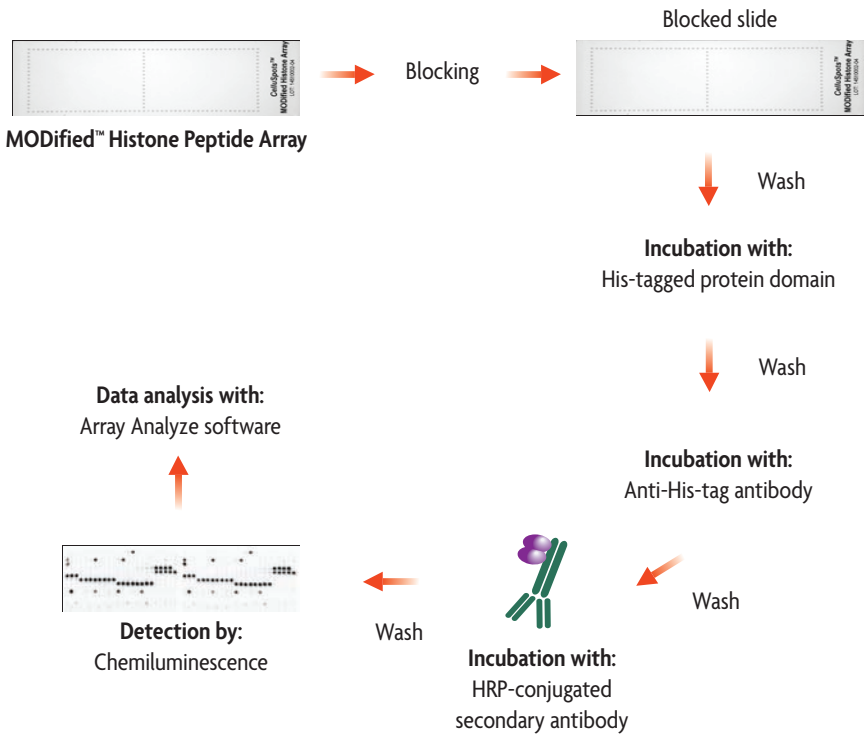
The Tudor domain folds into a β -sandwich flanked by one α -helix. Tudor domains are known to bind methylated lysine or arginine residues on Histone H3 and H4 and are believed to be involved in RNA binding, DNA damage response and chromatin modifications. Chromodomain proteins bind to methylated lysines on Histone H3 and are associated with the assembly of protein complexes that modify chromatin into condensed heterochromatin, such as transcriptional repressors HP-1, Polycomb (Pc) and the human retinoblastoma binding protein (RBP-1).

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 and its associated MODified Protein Domain Binding Kit provide a solution to this problem. The array contains 59 post-translational modifications in different combinations to allow researchers to screen a large panel of histone modifications in a single experiment. The results provide information on protein domain interactions with specific histone modifications and also reveals the influence that adjacent modifications may play on protein binding.

References

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4. Sims, R.J. and Reinberg, D. (2008) *Nat Rev Mol. Cell Biol*, 9 (10): 815-820.
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Flow chart of process



Flow Chart of MODified Protein Domain Binding Kit.

The MODified Histone Peptide Array is used in combination with the MODified Histone Protein Domain Binding Kit. The simple assay works like a Western blot. The array is incubated in blocking buffer. Then, the His-tagged protein domain of interest is added to the array followed by detection via an anti-His6-tag antibody and an HRP-conjugated secondary antibody. ECL detection is used to produce a chemiluminescent signal that is captured using a CCD camera or film. The array image is then analyzed using the Array Analyze software program to determine the spot intensity and identify protein domain binding partners.

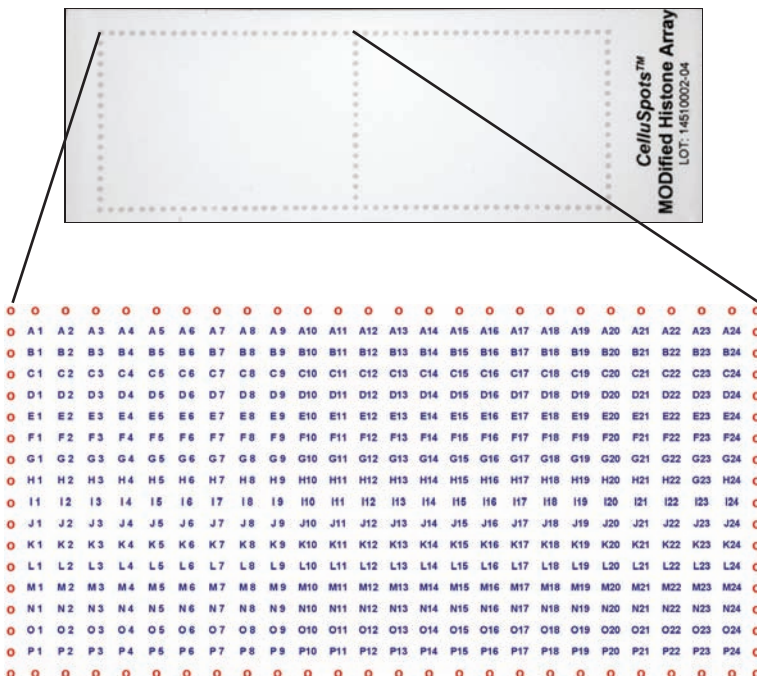
Kit Performance and Benefits

The MODified Histone Peptide Array can be used to screen antibodies, proteins and enzymes for interactions with histones and their post-translational modifications.

The array contains 59 different post-translational modifications for histone acetylation, methylation, phosphorylation and citrullination modifications on the N-terminal tails of histones H2A, H2B, H3 and H4. Each 19mer peptide may contain up to four modifications each. Five control spots are included on each array (locations P20-P24): biotin peptide, c-Myc tag, no histone peptide and two background spots containing a mixture of modifications that are present on the array.

The MODified Protein Domain Binding Kit includes Recombinant His-tagged G9a, a known histone methyltransferase that targets lysine 9 on Histone H3, as a positive control protein.

MODified Histone Peptide Array



MODified Histone Peptide Array and reference grid for histone peptide locations.

Histone peptides containing 384 different modification combinations are spotted in duplicate onto the glass slide. One spot is located on the left side of the slide and the duplicate spot is on the right side of the slide. The reference Excel file with the corresponding histone peptide content can be downloaded from Active Motif's website at www.activemotif.com/modified.

Example Analysis of Bromodomain BRD4 Protein Domain (333-460)

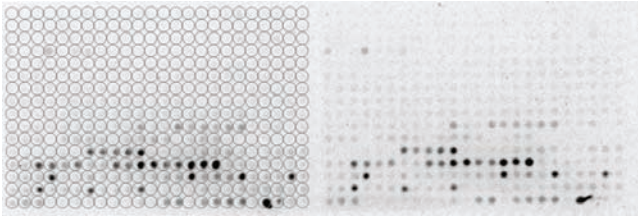
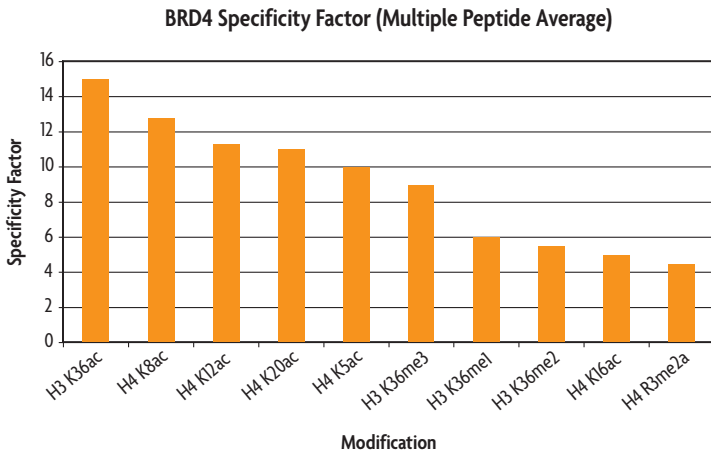


Image of ECL detection of Bromodomain-containing protein 4 (BRD4).

Recombinant His-tagged BRD4 (333-460) protein domain (Active Motif Catalog No. 31446) was incubated with the MODified Histone Peptide array in combination with a c-Myc antibody (Active Motif Catalog No. 39502) for control peptide P21 according to the MODified Histone Protein Domain Binding manual at a concentration of 180 nM for 2 hours. The anti-His6-tag antibody and c-Myc antibody were each used at a 1:6,000 dilution, followed by incubation with the anti-mouse HRP-conjugated antibody at a 1:2,500 dilution and ECL detection. The chemiluminescent signal from the array was captured with a CCD camera. The image shows the grid overlay from the Array Analyze software for spot identification on the left hand side of the array image.



Graphical analysis of the BRD4 protein domain.

Active Motif's Array Analyze Software was used to analyze spot intensity from the chemiluminescent image. The results were graphed as a specificity factor, which is the ratio of the average intensity of all spots containing the modification divided by the average intensity of all spots not containing the modification. The graph shows the top ten most reactive histone modifications based on the specificity factor values. The binding of BRD4 protein domain (333-460) showed specificity towards binding of acetylated histone lysine residues, confirming the usefulness of the MODified Peptide Array as a tool to screen for protein domain binding specificity.

Example Analysis of Positive Control G9a protein

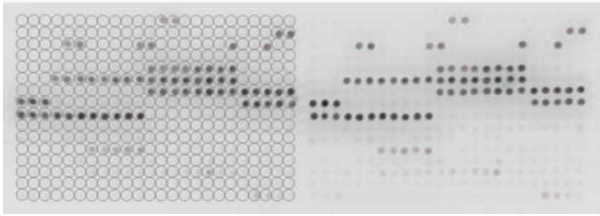
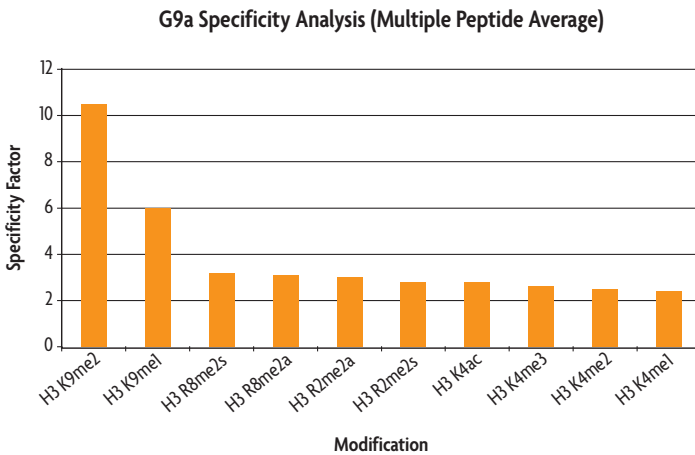


Image of ECL detection of positive control Recombinant His-G9a protein.

The positive control His-G9a protein was incubated with the MODified Histone Peptide array in combination with a c-Myc antibody (Active Motif Catalog No. 39502) for control peptide P21 according to the MODified Histone Protein Domain Binding manual at a concentration of 10 nM for 2 hours. The anti-His6-tag antibody and c-Myc antibody were each used at a 1:6,000 dilution, followed by incubation with the anti-mouse HRP-conjugated antibody at a 1:2,500 dilution and ECL detection. The chemiluminescent signal from the array was captured with a CCD camera. The image shows the grid overlay from the Array Analyze software for spot identification on the left hand side of the array image.



Graphical analysis of the positive control G9a protein.

Active Motif's Array Analyze Software was used to analyze spot intensity from the chemiluminescent image. The results were graphed as a specificity factor, which is the ratio of the average intensity of all spots containing the modification divided by the average intensity of all spots not containing the modification. The graph shows the top ten most reactive histone modifications based on the specificity factor values. G9a is a known histone methyltransferase associated with the specific mono- and di-methylation of lysine 9 on Histone H3. The data shown here confirm the association of G9a with its preferred histone modifications.

Kit Components and Storage

MODified Histone Peptide Arrays and the MODified Protein Domain Binding Kit are for research use only. Not for use in diagnostic procedures. All components are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity	Storage
10X Wash Buffer AM4	100 ml	4°C
Anti-mouse HRP-conjugated secondary antibody	10 µl	4°C
ECL Reagent A	50 µl	4°C
ECL Reagent B	2 x 13 ml	4°C
Blocking Buffer AM2	100 ml	-20°C
Interaction Buffer AM1	50 ml	-20°C
1 M DTT	100 µl	-20°C
Anti-His6-tag antibody	10 µl	-20°C
Recombinant His-G9a	20 µl	-80°C

Additional materials required but not provided

- MODified Histone Peptide Arrays
- His-tagged protein domain of interest
- Luminescent imaging system with CCD camera or X-ray film
- NUNC Well Rectangular Dishes (Catalog No. 267061) or suitable chamber for array incubation
- Active Motif's free Array Analyze Software program capable of analyzing MODified Histone Peptide Arrays (available for download at www.activemotif.com/modified)

Array specifications (not included in the MODified Protein Domain Binding Kit)

- Standard microscope slides (26x76 mm, white coating)
- 768 spots per slide (384 peptide-conjugate spots printed in duplicate)
- Spot-to-spot distance 1.2 mm
- Peptides are covalently bound to cellulose via C-terminus
- Arrays contain control peptides and location marks

Protocols

Buffer Preparation and Recommendations

Blocking Buffer AM2

Thaw the blocking buffer prior to starting the assay. Once thawed, the buffer should be stored at 4°C and used within 24 hours. Otherwise, the buffer should be re-frozen at -20°C.

Interaction Buffer AM1

Thaw the Interaction Buffer AM1 prior to starting the assay. Once thawed, the buffer should be stored at 4°C and used within one week. Otherwise, the buffer should be re-frozen at -20°C. Each array will need 10 ml of complete Interaction Buffer, which should be prepared fresh for each experiment by adding 1 µl of 1 M DTT to 10 ml Interaction Buffer AM1.

Preparation of 1 X Wash Buffer

Prepare the amount of 1X Wash Buffer required for the labeling assay as follows: For every 100 ml of 1X Wash Buffer required, dilute 10 ml 10X Wash Buffer AM4 with 90 ml distilled water (see the Quick Chart for Preparing Buffers). Mix gently to avoid foaming. The 1X Wash Buffer may be stored at 4°C for one week. The Tween 20 contained in the 10X Wash Buffer AM4 may form clumps, therefore it is necessary to completely resuspend any precipitates by incubating at 50°C for 2 minutes and mixing prior to use.

Sample Protein Domains

Transfer 5 ml of the complete Interaction Buffer containing DTT (prepared above) to a 15 ml conical tube. Dilute the His-tagged protein domain of interest in the Interaction Buffer. We recommend a range of 10 nM - 1 µM as a starting concentration. You may need to perform a titration of the protein domain to determine an optimal concentration for binding.

Note: The MODified Protein Domain Binding Kit is designed for use with His-tagged protein domains. If working with a GST or FLAG-tagged protein, please contact Active Motif's Technical Support team at tech_service@activemotif.com for recommendations on appropriate antibodies to use for detection.

Anti-His6-tag Antibody

This antibody is used to detect the His-tag on the protein domain of interest. Dilute the Anti-His6-tag antibody 1:3,000 in 1X Wash Buffer by adding 2 µl of antibody to 6 ml 1X Wash Buffer.

Anti-mouse HRP-conjugated secondary antibody

The anti-mouse HRP-conjugated secondary antibody should be diluted 1:2,500 by adding 2 µl of HRP-conjugated antibody to 5 ml 1X Wash Buffer.

Detecting Solution

To prepare the Detecting Solution, dilute ECL Reagent A into ECL Reagent B in a 15 ml conical tube. For a single array, add 1.5 µl of ECL Reagent A to 5 ml of ECL Reagent B. Vortex briefly to mix. Detecting Solution should be prepared just before use and kept protected from light. The

Detecting Solution should be added drop wise onto the array while gently tilting the array to ensure complete coverage of Detecting Solution over the full array (complete coverage may only require approximately 750 μ l of Detecting Solution). The array needs to remain wet during image capture. We recommend covering the array with clear plastic wrap or an acetate sheet during image capture to maintain the moisture.

Note: The MODified Histone Peptide Arrays have been validated for use on the LICOR® Odyssey Infrared Imaging System. To adapt the protocol for use with the infrared system, the appropriate infrared fluorescence secondary antibody and imaging system would be required in place of the anti-mouse HRP-conjugated secondary antibody and detecting solution.

Positive Control Recombinant His-G9a (Optional)

Recombinant His-G9a protein is provided as a positive control for the MODified Protein Domain Binding Kit. G9a is a histone lysine methyltransferase that specifically targets lysine 9 on Histone H3 for mono- and dimethylation. Due to the number of peptides containing Histone H3 lysine 9 on the array, it is not recommended to combine this positive control protein with your protein domain of interest. The Recombinant His-G9a protein is provided as an optional control that can be used to confirm the activity of your assay reagents, but should be tested on a separate MODified Histone Peptide Array than your protein of interest. Enough protein is supplied to run a single positive control experiment. The Recombinant His-G9a is provided ready to use. Add 20 μ l G9a protein to 5 ml complete Interaction Buffer and add to a separate array.

Note: See the Control Peptides section below and Appendix Section F for details regarding the use of a positive control antibody in combination with the sample protein domain of interest on the same array.

Control Peptides

The MODified Histone Peptide Array contains control spots (locations P20-P24), as referenced on the Excel file (www.activemotif.com/modified). The control c-Myc tag can be detected with an anti-c-Myc antibody (Catalog No. 39502). The control biotin peptide can be detected with either an anti-biotin antibody or a streptavidin-conjugated antibody. The array also contains a no histone negative control peptide and two spots for background levels. Control antibodies can be combined with the anti-His6-tag antibody for dual detection on the same array, as described in Appendix Section F: Detection of Control Peptides.

Quick Chart for Preparing Buffers

Reagents to prepare	Components	1 array	5 arrays
Blocking Buffer AM2	TOTAL REQUIRED	5 ml	25 ml
Interaction Buffer	Interaction Buffer AM1	10 ml	50 ml
	1 M DTT	1 μ l	5 μ l
	TOTAL REQUIRED	10 ml	50 ml
1X Wash Buffer	10X Wash Buffer AM4	10 ml	50 ml
	Distilled water	90 ml	450 ml
	TOTAL REQUIRED	100 ml	500 ml
ECL Reagent A	TOTAL REQUIRED	1.5 μl	7.5 μl
ECL Reagent B	TOTAL REQUIRED	5 ml	25 ml

Array Protocol

Read the entire protocol before use.

MODified Protein Domain Binding Kit Protocol

The protocol below is for screening protein domain interactions with histones and histone modifications using a chemiluminescent detection method. Prepare the Interaction Buffer, Blocking Buffer and 1X Wash Buffer as described above in the section Buffer Preparation and Recommendations. The protocol below is for the analysis of a single array. If screening multiple arrays at the same time, use the Quick Chart for Preparing Buffers to determine the total volumes needed.

It is very important to ensure that the entire surface of the MODified Histone Peptide Arrays are completely covered with liquid during all incubation and wash steps. Uneven distribution of liquid will result in inconsistent results. Do not touch or handle the membrane portion of the MODified Histone Peptide Array. Handle the array by the edges of the slide only.

Section A: Protein Domain Binding Interactions

1. Transfer 10 ml of thawed Blocking Buffer AM2 into 15 ml conical tube. Blocking Buffer AM2 can be kept at 4°C for up to 24 hours.
2. Immerse one array in 5 ml Blocking Buffer AM2.

Note: If working with multiple arrays at the same time, “4 Well Rectangular Dishes” from NUNC (Catalog No. 267061) can be used for the incubation and wash steps. The NUNC dish does not contain a lid, so it is recommended to use a low setting on an orbital shaker when performing incubation and wash steps. Other suitable containers may also be used.

3. Incubate on an orbital shaker for 1-4 hour(s) at room temperature, or overnight at 4°C.
4. Carefully pour off the buffer. Perform a quick rinse (30 seconds) with 5 ml 1X Wash Buffer. Then wash two times for 5 minutes each on an orbital shaker using 5 ml 1X Wash Buffer.
5. During the last wash step, prepare 10 ml of Interaction Buffer according to the Buffer Preparation and Recommendation Section on page 8.
6. Wash the array one time with 5 ml Interaction Buffer using an orbital shaker for 5 minutes at room temperature.
7. Add the His-tagged protein domain being studied and any necessary co-factors to 5 ml of Interaction Buffer. We recommend testing your protein domain in the range of 10 nM to 1 µM. Incubate the protein domain with the blocked array for 2 hours at room temperature. Set the orbital shaker to a low setting to prevent any cross contamination of protein domains if working with more than one array.

Note: If using the positive control His-G9a on the array, add 20 µl protein (10 nM final concentration) to 5 ml Interaction Buffer.

Section B: Addition of Anti-His Antibody

1. Perform a quick rinse (30 seconds) with 5 ml 1X Wash Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml 1X Wash Buffer.
2. During the last wash step, prepare the anti-His antibody dilution. Dilute the anti-His antibody 1:3,000 by adding 2 μ l anti-His tag antibody to 6 ml 1X Wash Buffer.
3. Add the 6 ml anti-His tag antibody to the array and incubate for 1 hour at room temperature on an orbital shaker.

Section C: Addition of HRP-Conjugated Secondary Antibody

1. Perform a quick rinse (30 seconds) with 5 ml 1X Wash Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml 1X Wash Buffer.
2. During the last wash step, prepare the secondary antibody dilution. Dilute the anti-mouse HRP-conjugated antibody 1:2,500 by adding 2 μ l anti-mouse HRP to 5 ml 1X Wash Buffer.
3. Add the 5 ml anti-mouse HRP-conjugated secondary antibody dilution to the array and incubate for 1 hour at room temperature on an orbital shaker.
4. Perform a quick rinse (30 seconds) with 5 ml 1X Wash Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml 1X Wash Buffer.
5. During the last wash step, prepare the Detecting Solution as stated in the Buffer Preparation and Recommendations section on page 8.

Section D: Detection

1. The Detecting Solution should be added drop wise onto the array while gently tilting the array to ensure complete coverage (approximately 750 μ l of Detecting Solution is required to completely cover the array). Let sit for 5 minutes at room temperature. The array needs to remain wet during image capture. We recommend covering the array with clear plastic wrap or an acetate sheet during image capture to maintain the moisture.

Note: If working with multiple arrays at the same time, we recommend going through the entire detection process and image capture with a single array and then moving on to the second array and so forth. Additional arrays can remain in the final wash from the secondary antibody until they are ready to be processed.

2. Use a CCD camera, or film, to capture images at multiple exposure times (e.g. 10 sec., 30 sec., 1 min., 2.5 min., 5 min. and 10 minutes). For weak protein domain binding interactions, an exposure of 10-15 minutes may be needed for sufficient detection. It is also recommended to take a white light image of the array in order to obtain orientation information for the analysis step.

Section E: Analysis

1. Save the image file as a .tif file and compare the image to the reference grid containing the histone peptide content. The .tif file will need to be resized to approximately 3298 pixels wide by 1161 pixels high for use in Active Motif's Array Analyze Software. Select constrain proportions to keep the scale consistent when resizing. (With constrain proportions selected only one value, either the width or height needs to be changed. The other value will auto correct.) Programs such as Adobe Photoshop or the freeware program, GIMP2 (<http://www.gimp.org/>) will allow you to open and resize .tif files. The image should also be saved with a mode value of 8 Bits/Channel.

The reference Excel file for the histone peptide content and associated grid location can be downloaded from our website at www.activemotif.com/modified.

2. Active Motif's free Array Analyze software is available for analysis of the MODified Histone Peptide Arrays. The software program will analyze the spot intensity of the interactions from the array and generate a graphical analysis of the histone peptide interactions. Information about spot intensity, averages and errors can be saved in Excel-compatible files to allow for individual analysis. The Array Analyze software is designed for use on PCs only. Please download the Array Analyze manual for information about the installation and operation of the program. Both the manual and the Array Analyze Software can be downloaded from our website at www.activemotif.com/modified.
3. (Optional) Use a spot densitometry program to determine the relative intensity of each peptide. Compare the image file to the reference grid containing the histone peptide content.

Section F: Detection of Control Peptides

This protocol is designed for researchers that want to include an antibody for detection of one of the control peptides (c-Myc or biotin) in combination with detection of their His-tagged protein domain of interest. We recommend detection of the c-Myc control spot (P21) using Active Motif's c-Myc antibody (Catalog No. 39502), as it can be used in combination with the supplied anti-mouse HRP-conjugated secondary antibody. If using another c-Myc antibody or an antibody to detect the biotin control peptide, it is necessary to use an antibody raised in mouse in order to utilize the anti-mouse HRP-conjugated secondary antibody for detection.

1. Dilute the antibody for the control peptide of interest (c-Myc or biotin) according to the Western blot dilution recommendations of the antibody supplier (For Cat# 39502, c-Myc antibody, we recommend using a 1:6,000 dilution). The antibody dilutions should be made into the same 6 ml 1X Wash Buffer used for the anti-His antibody such that the two antibodies are combined for simultaneous incubation (Section B, Step 2).
2. Add the 6 ml of combined anti-His and control spot antibody dilutions to the array and incubate for 1 hour at room temperature on an orbital shaker. Set the orbital shaker to a low setting to prevent any cross contamination of antibody solutions if working with more than one array.
3. Proceed to Section C: Addition of HRP-conjugated secondary antibody and continue with the instructions in the protocol.

Section G: Troubleshooting Guide

Problem/question	Possible cause	Recommendation
No signal or weak signal	Omission of key reagent	Check that all reagents have been added to the array in the correct order.
	Substrate is no longer active	Test conjugate and substrate for activity by mixing a small amount of HRP and detecting solution together.
	Enzyme inhibitor present	Sodium azide will inhibit peroxidase reactions (HRP-conjugates). Follow our recommendations to prepare buffers.
	Concentration of protein domain is too low	Titrate the amount of protein domain to use in the assay for optimal binding. We recommend a range of 10 nM to 1 μ M.
	CCD camera settings not optimal	Verify the filter settings on the CCD camera and make sure they are set to detect luminescence
High background	Concentration of protein domain is too high	Titrate the amount of protein domain to use in the assay for optimal binding. We recommend a range of 10 nM to 1 μ M.
	Non-specific protein binding	Some protein domain do not have specific histone binding partners. As a result, the protein may bind to a large number of peptides on the MODified Histone Peptide Array. This may appear as background signal due to the highly promiscuous nature of the protein domains. Confirm with published literature the expected histone binding sites for your protein domain of interest.
Uneven detection or inconsistent replicates	Not enough solution used for incubation and wash steps	It is very important to ensure that the entire surface of the MODified Histone Peptide Arrays are completely covered with liquid during all incubation and wash steps. Uneven distribution of liquid will result in inconsistent results. If using large containers for incubation and wash steps it may be necessary to increase the volume of solution used.
	Incubation steps performed on an uneven surface	It is very important to ensure that the entire surface of the MODified Histone Peptide Arrays are completely covered with liquid during all incubation and wash steps. Uneven distribution of liquid will result in inconsistent results. Ensure that the array is placed on a flat, level surface during all incubation and wash steps.
	Array dried out during image capture	During long exposure times it is possible for the Detection Solution to be completely absorbed by the array, causing the array to dry out. Place a sheet of clear plastic wrap or an acetate sheet over the array in order to keep the array wet during image capture.
Can the MODified Histone Peptide Arrays be stripped and re-used?		No. The MODified Histone Peptide arrays are suitable for one use only. Standard stripping procedures do not adequately remove the existing bound antibody and harsh conditions can cleave the peptide bond to the array.

Problem/question	Possible cause	Recommendation
<p>What if my protein domain of interest contains a GST or FLAG tag instead of a His tag?</p>		<p>The MODified Protein Domain Binding Kit is designed to be used with His-tagged proteins and therefore includes an anti-His-tag antibody for detection. To modify the assay for use with a protein containing a GST, FLAG or other tag you would need to identify a suitable tag specific antibody for substitution in the kit and an associated HRP-conjugated secondary antibody. Please contact our technical support department for recommendations on appropriate antibodies to use for this application.</p>
<p>What are the chemical stability limits of the arrays?</p>		<p>The arrays are stable at the physiological pH values. They are sensitive to mechanical stress and should not be touched or wiped. Furthermore, the arrays should not be treated with strong acids and bases, since the peptide bond and/or the linker between the peptide and cellulose can be cleaved. The treatment with organic solvents like ethanol should also be avoided. Several salt concentrations have been tested for blocking and wash buffers, and the arrays are robust in this respect.</p>

Section H. Related Products

Histone ELISAs	Format	Catalog No.
Histone H3 monomethyl Lys4 ELISA	1 x 96 rxns	53101
Histone H3 dimethyl Lys4 ELISA	1 x 96 rxns	53112
Histone H3 trimethyl Lys4 ELISA	1 x 96 rxns	53113
Histone H3 dimethyl Lys9 ELISA	1 x 96 rxns	53108
Histone H3 trimethyl Lys9 ELISA	1 x 96 rxns	53109
Histone H3 monomethyl Lys27 ELISA	1 x 96 rxns	53104
Histone H3 trimethyl Lys27 ELISA	1 x 96 rxns	53106
Histone H3 phospho Ser10 ELISA	1 x 96 rxns	53111
Histone H3 phospho Ser28 ELISA	1 x 96 rxns	53100
Total Histone H3 ELISA	1 x 96 rxns	53110

Recombinant Histones	Format	Catalog No.
Recombinant Histone H2A	50 µg	31251
Recombinant Histone H2B	50 µg	31252
Recombinant Histone H3 (C110A)	100 µg	31207
Recombinant Histone H3 biotinylated	25 µg	31271
Recombinant Histone H3K4me1	50 µg	31208
Recombinant Histone H3K4me2	50 µg	31209
Recombinant Histone H3K4me3	50 µg	31210
Recombinant Histone H3R8me2a	25 µg	31276
Recombinant Histone H3 monomethyl Lys9	50 µg	31211
Recombinant Histone H3 dimethyl Lys9	50 µg	31212
Recombinant Histone H3 trimethyl Lys9	50 µg	31213
Recombinant Histone H3 monomethyl Lys27	50 µg	31214
Recombinant Histone H3 dimethyl Lys27	50 µg	31215
Recombinant Histone H3 trimethyl Lys27	50 µg	31216
Recombinant Histone H3 monomethyl Lys36	50 µg	31217
Recombinant Histone H3 dimethyl Lys36	50 µg	31218
Recombinant Histone H3 trimethyl Lys36	50 µg	31219
Recombinant Histone H3 monomethyl Lys79	50 µg	31220
Recombinant Histone H3 dimethyl Lys79	50 µg	31221
Recombinant Histone H3 trimethyl Lys79	50 µg	31222
Recombinant Histone H4	50 µg	31223
Recombinant Histone H4 monomethyl Lys20	50 µg	31224
Recombinant Histone H4 dimethyl Lys20	50 µg	31225
Recombinant Histone H4 trimethyl Lys20	50 µg	31226

Active Motif also offers a growing list of application validated antibodies, including antibodies for histones and histone modifications, transcription factor antibodies, DNA methylation-related antibodies and ChIP validated antibodies. For a complete list go to www.activemotif.com/abs

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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