MODified™ Histone Peptide Array

(version B3)

Catalog Nos. 13001 & 13005

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Overview

The MODified™ Histone Peptide Array* is a valuable research tool that can be used to screen antibodies, proteins and enzymes for interactions with histones and their post-translational modifications. Each array contains 384 different histone modification combinations in duplicate for acetylation, methylation, phosphorylation and citrullination modifications on the N-terminal tails of histones H2A, H2B, H3 and H4.

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 Histone Peptide Array can be used to screen antibodies for cross-reactivity or to study protein and enzyme interactions. The array itself contains peptide-cellulose-conjugates spotted onto the planar surface of a standard microscope slide in a three-dimensional layer. This high peptide density enables even protein-interaction sites with low binding constants to be detected.

The simple array protocol works like a Western blot. Either ECL-based or colorimetric detection systems can be used. The image is then captured using film or a CCD camera; no special equipment is needed. Active Motif's free Array Analyze Software can be used to analyze the intensity of the spots and generate a graphical analysis of the histone peptide modification interactions. Information about spot intensity, averages and errors can be saved in Excel-compatible files. For added convenience, up to three individual modifications can be displayed in superposition to the experimental data enabling better visualization of the effects of neighboring modifications.

product	format	catalog no.
MODified™ Histone Peptide Array	1 array 5 arrays	13001 13005
MODified™ Array Labeling Kit	5 rxns	13006
MODified™ Protein Domain Binding Kit	5 rxns	13007

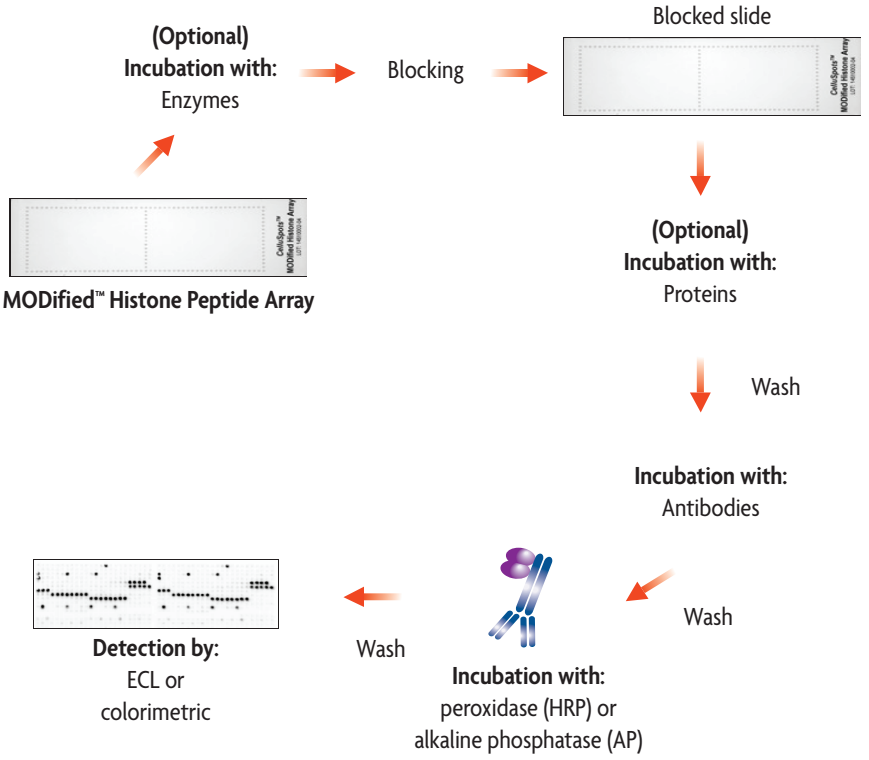
Related Products

Active Motif's MODified™ Array Labeling Kit and MODified™ Protein Domain Binding Kit can be purchased in conjunction with the MODified Histone Peptide Arrays. The MODified Array Labeling Kit (Catalog No. 13006) contains buffers, control antibody, secondary antibodies and ECL reagents for chemiluminescent detection of five MODified Histone Peptide Arrays.

The MODified Protein Domain Binding Kit (Catalog No. 13007) is designed to screen protein domains for interactions with histones and their post-translational modifications and contains buffers and reagents for chemiluminescent detection of five MODified Histone Peptide Arrays. A positive control G9a tudor domain protein is also included.

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

Flow chart of process

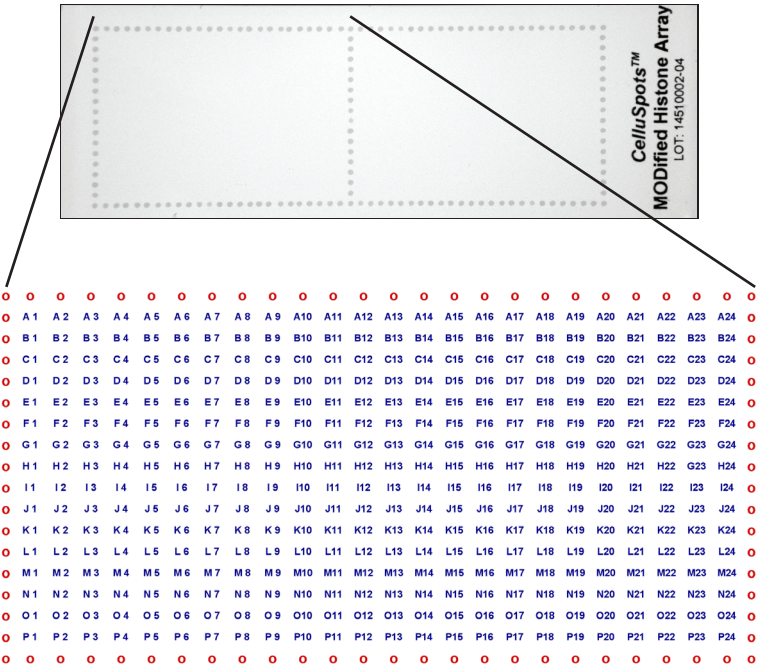


Kit Performance and Benefits

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.

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 Histone H3 trimethyl Lys9 pAb (Catalog No. 39161)

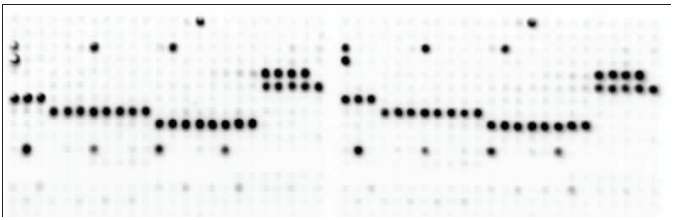
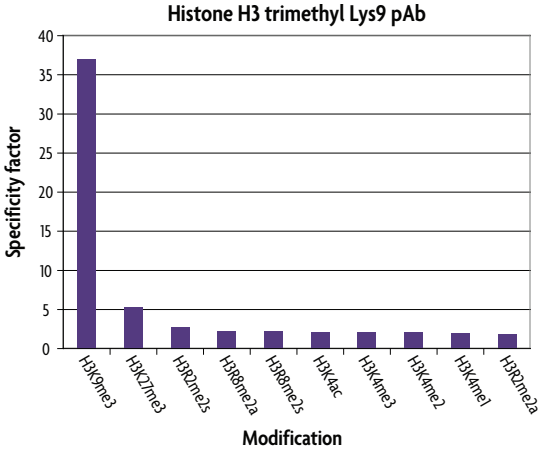


Image of ECL detection of Histone H3 trimethyl Lys9 (H3K9me3) pAb.

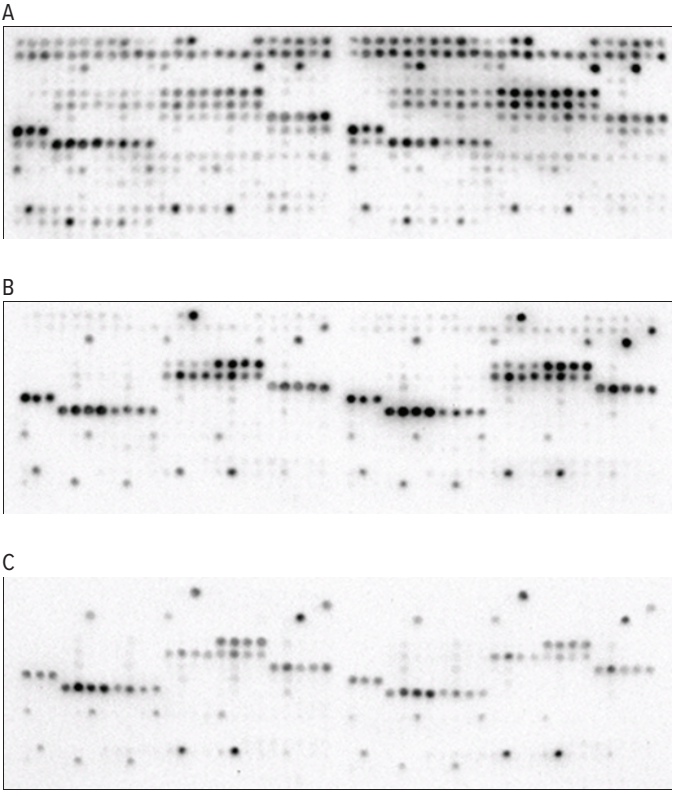
Active Motif's Histone H3 trimethyl Lys9 pAb (Catalog No. 39161) was used at 1:2,000 dilution on the MODified Histone Peptide Array. Anti-rabbit HRP secondary antibody was used at 1:2500 dilution, followed by ECL detection and image capture with a CCD camera.



Graphical analysis of Histone H3 trimethyl Lys9 (H3K9me3) pAb cross-reactivity.

Active Motif's Array Analyze Software was used to analyze spot intensity from the ECL camera image. The results were graphed as a specificity factor, which is the ratio of the average intensity of all spots containing the mark divided by the average intensity of all spots not containing the mark. The results show Active Motif's Histone H3 trimethyl Lys9 pAb (Catalog No. 39161) has very little cross-reactivity with other histone modifications.

Example Analysis of Histone Methyltransferase G9a and G9a mut (Catalog Nos. 31327 & 31328)



Images of ECL detection of histone methyltransferase G9a treated arrays.

A) Twenty-five µM of Active Motif's G9a methyltransferase (Catalog No. 31327) was incubated with the MODified Histone Peptide Array overnight in the presence of 50 mM Tris, pH 9.0, 5 mM $MgCl_2$, 4 mM DTT and 1 mM AdoMet.

B) Twenty-five µM of Active Motif's G9a mutant H904K methyltransferase (Catalog No. 31328) was incubated with the MODified Histone Peptide Array overnight in the presence of 50 mM Tris, pH 9.0, 5 mM $MgCl_2$, 4 mM DTT and 1 mM AdoMet.

C) No enzyme control. The methylation status for all three arrays (A-C) was then detected using Histone H3 dimethyl Lys9 pAb at 1:2,000 dilution. Anti-rabbit HRP secondary antibody was used at 1:2500 dilution, followed by ECL detection and image capture with a CCD camera. Novel methylation sites were observed on array A, which was treated with the wild-type G9a methyltransferase, showing the ability of the G9a histone methyltransferase to use the peptide sequences on the array as substrates for histone methylation. Example analysis of the spot intensity from the array incubated with histone methyltransferase G9a are shown on the next page.

Example Analysis of Histone Methyltransferase G9a

Peptide No.	Intensity avg.	Peptide sequence	Mod1	Mod2	Mod3	Mod4
A 1	0.3320	A R T K Q T A R K S T G G K A P R K Q	unmod			
A13	0.6268	A R T K Q T A R Kme1 S T G G K A P R K Q	K9me1			
A14	0.8333	A R T K Q T A R Kme2 S T G G K A P R K Q	K9me2			
A15	0.0564	A R T K Q T A R Kme3 S T G G K A P R K Q	K9me3			
B23	0.3735	A R T K Q T A Rme2a Kme1 S T G G K A P R K Q	R8me2s	K9me1		
B24	0.7933	A R T K Q T A Rme2a Kme2 S T G G K A P R K Q	R8me2s	K9me2		
C 1	0.0644	A R T K Q T A Rme2a Kme3 S T G G K A P R K Q	R8me2s	K9me3		
C 5	0.3431	A R T K Q T A Rme2a Kme1 S T G G K A P R K Q	R8me2a	K9me1		
C 6	0.6484	A R T K Q T A Rme2a Kme2 S T G G K A P R K Q	R8me2a	K9me2		
C 7	0.1093	A R T K Q T A Rme2a Kme3 S T G G K A P R K Q	R8me2a	K9me3		
C11	0.2053	A R T K Q T A Cit Kme1 S T G G K A P R K Q	R8Citr	K9me1		
C12	0.3189	A R T K Q T A Cit Kme2 S T G G K A P R K Q	R8Citr	K9me2		
C13	0.0819	A R T K Q T A Cit Kme3 S T G G K A P R K Q	R8Citr	K9me3		
C17	0.0440	A R T K Q T A R Kme1 pS T G G K A P R K Q	K9me1	S10P		
C18	0.0879	A R T K Q T A R Kme1 S pT G G K A P R K Q	K9me1	T11P		
C19	0.8930	A R T K Q T A R Kme1 S T G G Kac A P R K Q	K9me1	K14ac		
C20	0.0797	A R T K Q T A R Kme2 pS T G G K A P R K Q	K9me2	S10P		
C21	0.1319	A R T K Q T A R Kme2 S pT G G K A P R K Q	K9me2	T11P		
C22	0.8570	A R T K Q T A R Kme2 S T G G Kac A P R K Q	K9me2	K14ac		
C23	0.0733	A R T K Q T A R Kme3 pS T G G K A P R K Q	K9me3	S10P		
C24	0.0529	A R T K Q T A R Kme3 S pT G G K A P R K Q	K9me3	T11P		
D 1	0.0568	A R T K Q T A R Kme3 S T G G Kac A P R K Q	K9me3	K14ac		
G20	0.4413	A Rme2s T Kme1 Q T A Rme2s Kme2 S T G G K A P R K Q	R2me2s	K4me1	R8me2s	K9me2
H 4	0.0668	A Rme2s T Kme1 Q T A Rme2s Kme3 S T G G K A P R K Q	R2me2s	K4me1	R8me2s	K9me3

Intensity analysis of histone methyltransferase G9a incubation with the MODified Histone Peptide Array.

The MODified Histone Peptide array was incubated in the presence of histone methyltransferase G9a (Catalog No. 31327) using the conditions stated on the previous page and detected with Histone H3 dimethyl Lys9 pAb. The average spot intensity values for selected modification combinations are shown. The trimethyl Lys9 modifications have very low intensity as they are not suitable substrates for G9a methylation, nor are they detected by the dimethyl antibody. However, the unmodified, mono- and dimethyl Lys9 sites show good intensity values since the G9a histone methyltransferase was able to use each of these as substrates and the dimethyl antibody detected this new methylation as well as the dimethyl peptides that were not modified. The impact of neighboring modifications is apparent when S10P or T11P exist on the same peptide as monomethyl or dimethyl Lys9, as these phosphorylation sites greatly reduce the intensity values.

Kit Components and Storage

MODified Histone Peptide Arrays 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
MODified™ Histone Peptide Array	1 array 5 arrays	4°C for up to 3 months or -20°C for up to 6 months

Additional materials required

- TTBS Buffer (10 mM Tris-HCl, pH 7.4, 0.05% Tween 20 and 150 mM NaCl)
- Non-fat dried milk
- Primary antibody, protein or enzyme for analysis
- Appropriate enzyme activity buffer or protein binding buffer if the arrays are to be used for protein or enzyme studies and a related primary antibody for recognition
- Secondary antibody conjugated to horseradish peroxidase or alkaline phosphatase
- Detection reagents (ECL or colorimetric)
- Luminescent or colorimetric imaging system with CCD camera or X-ray film
- Optional: 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

- 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

Buffer Preparation and Recommendations

TTBS Buffer

Prepare 100 ml TTBS Buffer containing 10 mM Tris-HCl pH 7.4, 0.05% Tween 20 and 150 mM NaCl. This buffer will be used to prepare the Blocking Solution and for all the wash steps. This buffer can be prepared in advance and stored at room temperature.

Blocking Solution

Prepare 10 ml TTBS Buffer containing 5% non-fat dried milk for blocking and antibody dilutions. Blocking solution should be prepared just before use, or prepared and stored at 4°C for up to 24 hours.

Sample Enzymes

Prepare 3 ml of appropriate enzyme activity buffer. Dilute the enzyme for analysis in the activity buffer, making sure to include any appropriate co-factors (*e.g.* include methyl donor AdoMet (also known as SAM) with a histone methyltransferase enzyme). Use the appropriate antibody (*e.g.* anti-H3 Lys9 dimethyl antibody) and corresponding species specific conjugated secondary antibody for detection. Follow the recommendations below for preparing antibody dilutions.

Sample Proteins

Prepare 3 ml of appropriate protein binding buffer. Dilute the protein for analysis in the binding buffer. Use the appropriate antibody and corresponding species specific conjugated secondary antibody for detection. Follow the recommendations below for preparing antibody dilutions.

Sample Antibodies

Antibodies should be diluted in 3 ml Blocking Solution. As a general guideline we suggest using monoclonal antibodies at a 1:2000 dilution. For polyclonal antibodies, a 2-fold dilution over the amount recommended by the antibody provider for Western blot is usually sufficient.

Use a corresponding species specific secondary antibody conjugated to either horseradish peroxidase (HRP) or alkaline phosphatase (AP). Dilute conjugated secondary antibody as recommended by the antibody provider for Western blot in 3 ml Blocking Solution (1:2,500 may be a suitable dilution for most secondary antibodies).

Detection Reagents

Follow the recommendations of the manufacturer for the appropriate detection conditions. The Detection Reagent should be added drop wise onto the array while tilting the array to ensure complete coverage of Detection Reagent over the full 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 biotin spot can be detected with either an anti-biotin antibody or a streptavidin-conjugated antibody, while the c-myc control can be detected with an anti-c-myc antibody. The desired control antibody can be combined with the primary antibody being tested, or added sequentially as described in Appendix A. The array also contains a negative control peptide and two spots for background levels.

Note: The non-fat dried milk used to generate the Blocking Solution may contain trace biotin molecules which can sequester the biotin or streptavidin detecting antibody and reduce signal intensity. For detection of the biotin control peptide on the MODified Histone Peptide Array, we recommend diluting the anti-biotin or streptavidin-conjugated antibody in TTBS Buffer instead of Blocking Solution.

Quick Chart for Preparing Buffers*

Sample Type	Reagents to prepare	Components	1 array	5 arrays
Enzyme or Protein	Activity or Binding Buffer	Provided by the customer	**3 ml**	**15 ml**
	Blocking Solution	**TOTAL REQUIRED**	**10 ml**	**50 ml**
	TTBS Buffer	**TOTAL REQUIRED**	**90 ml**	**450 ml**
Antibody	Blocking Solution	**TOTAL REQUIRED**	**10 ml**	**50 ml**
	TTBS Buffer	**TOTAL REQUIRED**	**70 ml**	**350 ml**

*If performing sequential antibody addition of sample antibody and control antibodies, please refer to the calculations listed in Appendix A for preparing buffer volumes.

Read the entire protocol before use.

MODified Histone Peptide Array Protocol

The protocol below is for screening antibody cross-reactivity or protein and enzyme interactions with histones and histone modifications. Either an ECL or colorimetric detection method can be used. Prepare the TTBS Buffer and Blocking Solution 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. For enzyme analysis begin with Step 1; for protein studies start with Step 2; for antibody studies proceed to Step 3.

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.

Step 1: Enzyme Interactions

1. Prepare the appropriate activity buffer for the enzyme being studied.
2. Immerse one array in 3 ml activity buffer.

   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 20 minutes at room temperature.
4. Add the enzyme being studied and any necessary co-factors to the array. Incubate for the desired time.
5. Carefully pour off the buffer. Perform a quick rinse (30 seconds) with 5 ml TTBS buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml TTBS buffer.
6. Prepare 10 ml of Blocking Solution.
7. Immerse one array in 3 ml Blocking Solution.
8. Incubate on an orbital shaker for 1-4 hour(s) at room temperature, or overnight at 4°C.
9. Perform a quick rinse (30 seconds) with 5 ml TTBS buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml TTBS buffer.
10. During the last wash step, prepare appropriate antibody dilutions.
11. Dilute the primary antibody in 3 ml Blocking Solution. See the dilution suggestions in the Buffer Preparation and Recommendations section on page 8.

Note: To detect the c-Myc control peptide on the array, combine a c-Myc antibody with the sample antibody in 3 ml Blocking Solution. Make sure to use appropriate species-specific secondary antibody for detection of both the sample and control antibodies. Due to the presence of biotin in the non-fat dried milk used to make the Blocking Solution, we do not recommend performing anti-biotin or streptavidin-conjugated antibody incubations in Blocking Solution. For sequential detection of the control and sample antibodies, please refer to Appendix A.

12. Add the primary antibody dilution to the array and incubate for 1 hour at room temperature, or overnight at 4°C 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.
13. Perform a quick rinse (30 seconds) with 5 ml TTBS buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml TTBS buffer.
14. During the last wash step, prepare the secondary antibody dilution in 3 ml Blocking Solution. Proceed to Step 4.

Step 2: Protein Interactions

1. Prepare 10 ml of Blocking Solution.
2. Immerse one array in 3 ml Blocking Solution.

   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 TTBS buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml TTBS buffer.
5. During the last wash step, prepare 3 ml of the appropriate protein binding buffer.
6. Add the protein being studied and any necessary co-factors to the protein binding buffer. Incubate with the blocked array for the desired time.
7. Perform a quick rinse (30 seconds) with 5 ml TTBS buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml TTBS buffer.
8. During the last wash step, prepare appropriate antibody dilutions.
9. Dilute the primary antibody in 3 ml Blocking Solution. See the dilution suggestions in the Buffer Preparation and Recommendations section on page 8.

   Note: To detect the c-Myc control peptide on the array, combine a c-Myc antibody with the sample antibody in 3 ml Blocking Solution. Make sure to use appropriate species-specific secondary antibody for detection of both the sample and control antibodies. Due to the presence of biotin in the non-fat dried milk used to make the Blocking Solution, we do not recommend performing anti-biotin or

streptavidin-conjugated antibody incubations in Blocking Solution. For sequential detection of the control and sample antibodies, please refer to Appendix A.

10. Add the primary antibody dilution to the array and incubate for 1 hour at room temperature, or overnight at 4°C 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.
11. Perform a quick rinse (30 seconds) with 5 ml TTBS buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml TTBS buffer.
12. During the last wash step, prepare the secondary antibody dilution in 3 ml Blocking Solution. Proceed to Step 4.

Step 3: Antibody Studies

1. Prepare 10 ml of Blocking Solution.
2. Immerse one array in 3 ml Blocking Solution.

   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 TTBS buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml TTBS buffer.
5. During the last wash step, prepare appropriate antibody dilutions.
6. Dilute the primary antibody in 3 ml Blocking Solution. See the dilution suggestions in the Buffer Preparation and Recommendations section on page 8.

   Note: To detect the c-Myc control peptide on the array, combine a c-Myc antibody with the sample antibody in 3 ml Blocking Solution. Make sure to use appropriate species-specific secondary antibody for detection of both the sample and control antibodies. Due to the presence of biotin in the non-fat dried milk used to make the Blocking Solution, we do not recommend performing anti-biotin or streptavidin-conjugated antibody incubations in Blocking Solution. For sequential detection of the control and sample antibodies, please refer to Appendix A.

7. Add the primary antibody dilution to the array and incubate for 1 hour at room temperature, or overnight at 4°C 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.
8. Perform a quick rinse (30 seconds) with 5 ml TTBS buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml TTBS buffer.
9. During the last wash step, prepare the secondary antibody dilution in 3 ml Blocking Solution. Proceed to Step 4.

Step 4: Addition of Conjugated Secondary Antibody

1. Add the conjugated secondary antibody dilution 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.
2. Perform a quick rinse (30 seconds) with 5 ml TTBS buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml TTBS buffer.
3. During the last wash step, prepare the detection solution according to the manufacturer's recommendations. Approximately 1.5 ml of detecting solution is sufficient to cover the array.

Step 5: Detection

1. Add the detection solution to the array. The Detection Reagent should be added drop wise onto the array while gently tilting the array to ensure complete coverage. 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.
2. For ECL detection, 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 antibodies 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. For colorimetric detection a standard camera or scanner can be used to capture images.

Step 6: 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 specific dimensions, as stated in the Array Analyze manual, for use in Active Motif's Array Analyze Software. Programs such as Adobe Photoshop or the freeware program, GIMP2 (http://www.gimp.org/) will allow you to open and resize .tif files.

   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.

Appendix

Section A: Sequential Antibody Incubation

This protocol is designed for researchers that prefer to perform separate incubation of sample antibody and control antibody. This protocol will require the preparation of additional Blocking Solution and TTBS Buffer than the Standard Protocol. Refer to the Quick Chart below for buffer preparation.

1. If using enzyme samples, follow Step 1, instructions # 1-11. For instruction # 11, add the appropriate antibody dilution for detection of the enzyme reaction in 3 ml Blocking Solution. Do not combine the control antibody into the mixture.

   If using protein samples, follow Step 2, instructions # 1-9. For instruction # 9, add the appropriate antibody dilution for detection of the protein interaction in 3 ml Blocking Solution. Do not combine the control antibody into the mixture.

   If using antibody samples, follow Step 3, instructions # 1-6. For instruction # 6, add the appropriate sample antibody dilution in 3 ml Blocking Solution. Do not combine the control antibody into the mixture.
2. Add the sample antibody dilution to the array and incubate for 1 hour at room temperature, or overnight at 4°C 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. Perform a quick rinse (30 seconds) with 5 ml TTBS Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml TTBS Buffer.
4. During the last wash step, prepare the control antibody dilution in 3 ml Blocking Solution.
5. 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.
6. Perform a quick rinse (30 seconds) with 5 ml TTBS Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml TTBS Buffer.
7. During the last wash step, prepare the secondary antibody dilution in 3 ml Blocking Solution. Add the species specific HRP-conjugated secondary antibody needed for detection of the sample and control antibodies. If using secondary antibodies of different species, they may be combined together into a single incubation step, or also incubated sequentially with washing between addition of each antibody.
8. 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.
9. Perform a quick rinse (30 seconds) with 5 ml TTBS Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml TTBS Buffer.

10. During the last wash step, prepare the Detecting Reagent as stated in the Buffer Preparation and Recommendations section on page 8. Approximately 1.5 ml of Detecting Reagent is sufficient to cover the entire array.
11. Continue on with Step 5 and Step 6 of the Standard Protocol for detection and analysis of the arrays.

Quick Chart for Preparing Buffers for Sequential Incubations

Sample Type	Reagents to prepare	Components	1 array	5 arrays
Enzyme or Protein	Activity or Binding Buffer	Provided by the customer	**3 ml**	**15 ml**
	Blocking Solution	**TOTAL REQUIRED**	**18 ml**	**80 ml**
	TTBS Buffer	**TOTAL REQUIRED**	**150 ml**	**650 ml**
Antibody	Blocking Solution	**TOTAL REQUIRED**	**18 ml**	**80 ml**
	TTBS Buffer	**TOTAL REQUIRED**	**110 ml**	**550 ml**

Section B: 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 or AP and detecting solution together.
	Enzyme inhibitor present	Sodium azide will inhibit peroxidase reactions (HRP-conjugates). Follow our recommendations to prepare buffers.
	Concentration of antibodies is too low	Increase the amount of antibody used with the array.
	CCD camera settings not optimal	Verify the filter settings on the CCD camera and make sure they are set to detect luminescence or colorimetric readouts based on the type of detection being used.
High background	Concentration of antibodies is too high	For monoclonal antibodies we suggest a 1:2000 dilution. For polyclonal antibody, we suggest a 2-fold dilution over the recommendations of the antibody provider for Western blot detection. If these recommendations were followed and there is still high background, we suggest diluting the antibody 5 to 10-fold for future testing.
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.
What are the chemical stability limits of the arrays?		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.

Section 4: Technical Services

If you need assistance at any time, please call or send an email to Active Motif Technical Service at one of the locations listed below.

Active Motif North America

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