ACTIVE MOTIF®

Enabling Epigenetics Research

MODified™ Array Labeling Kit

Catalog No. 13006

(Version A2)

Revision	Date	Description of Change
A2	September, 2023	C-Myc control monoclonal antibody was changed to AbFlex® Myc-Tag antibody (rAb) (Cat# 91203). The dilution for use of this recombinant has been updated.

Contents

Overview

The MODified™ Array Labeling Kit contains the buffers and reagents needed for easy labeling and chemiluminescent detection of the MODified™ Histone Peptide Arrays. The MODified™ Array Labeling Kit contains blocking buffer, wash buffer, rabbit and mouse HRP-conjugated secondary antibodies and ECL reagents for chemiluminescent detection. For added convenience, a positive control Myc-Tag recombinant antibody is included to recognize the arrays' control Myc-Tag.

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.

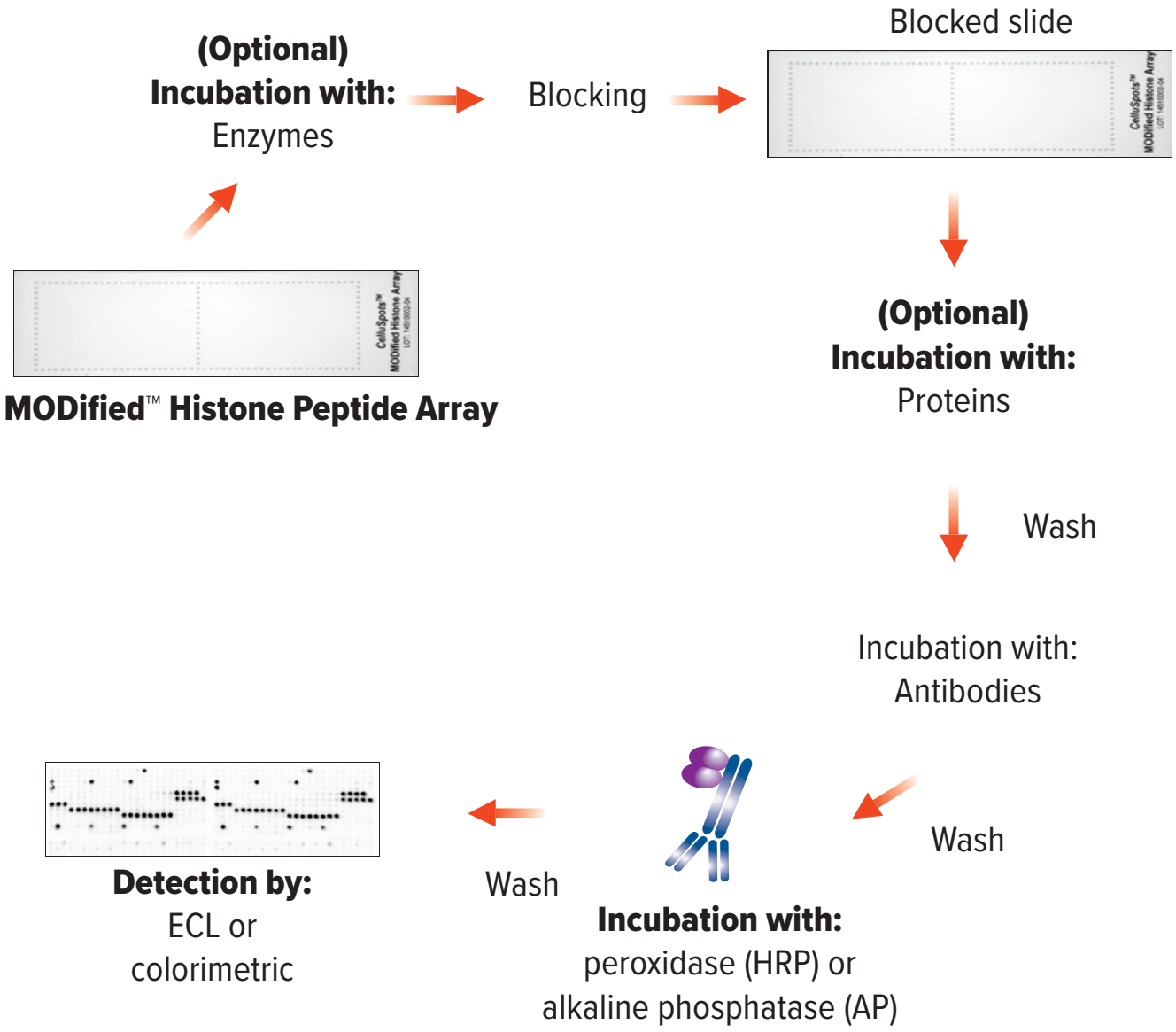
Following labeling and detection, the image is then captured using film or a CCD camera; no special equipment is needed. Active Motif's free Array Analyse Software can be used to analyze the intensity of the spots and generate a graphical analysis of the histone peptide modification interactions. Information 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.

The MODified™ Histone Peptide Arrays are available individually, or in packs of five. The arrays are not included in the MODified Array Labeling Kit and must be purchased separately.

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

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

Flow Chart of Process



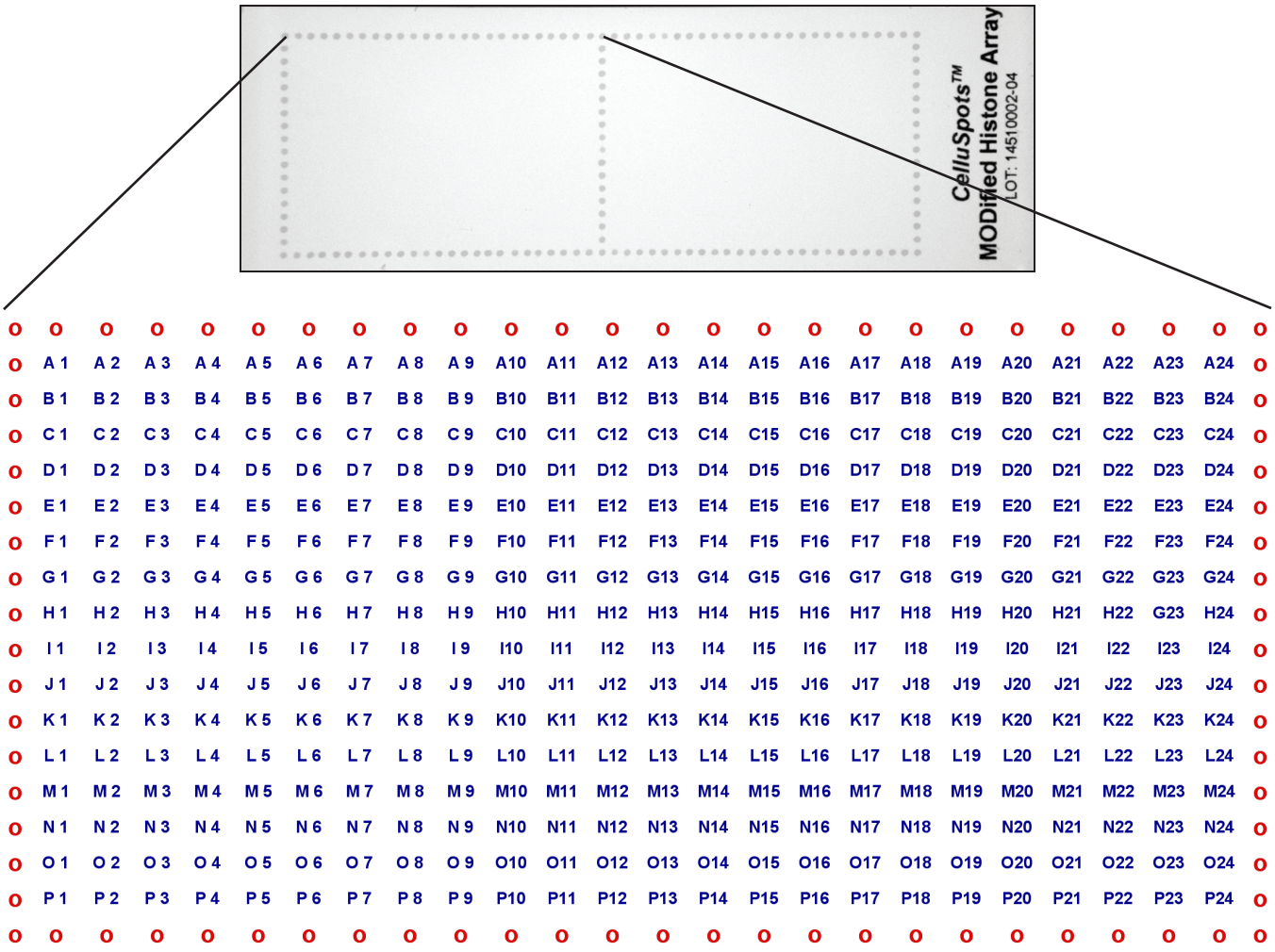
Kit Performance and Benefits

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, Myc-Tag, no histone peptide and two background spots containing a mixture of modifications that are present on the array.

The MODified™ Array Labeling Kit includes an antibody to recognize the Myc-Tag control spot as well as anti-rabbit and anti-mouse HRP-conjugated secondary antibodies for detection.

MODified™ Histone Peptide Array



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o	B 1	B 2	B 3	B 4	B 5	B 6	B 7	B 8	B 9	B10	B11	B12	B13	B14	B15	B16	B17	B18	B19	B20	B21	B22	B23	B24	o
o	C 1	C 2	C 3	C 4	C 5	C 6	C 7	C 8	C 9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	o
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o	E 1	E 2	E 3	E 4	E 5	E 6	E 7	E 8	E 9	E10	E11	E12	E13	E14	E15	E16	E17	E18	E19	E20	E21	E22	E23	E24	o
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o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o

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 activemotif.com/MODified.

Example Analysis of Histone H3 trimethyl Lys9 pAb, Cat. No. 39161

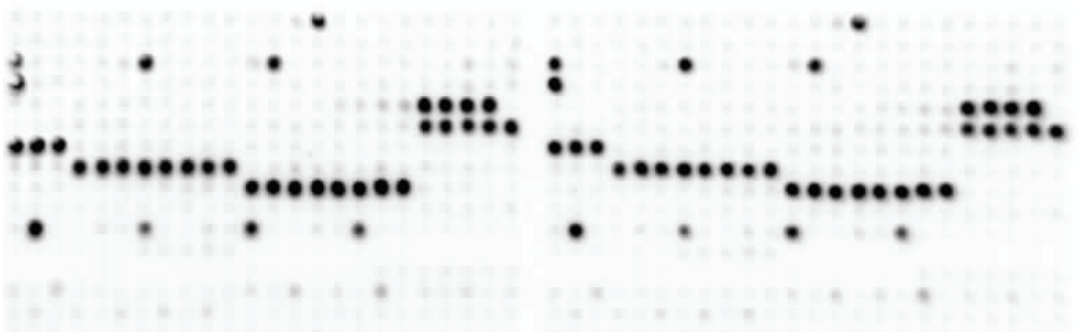
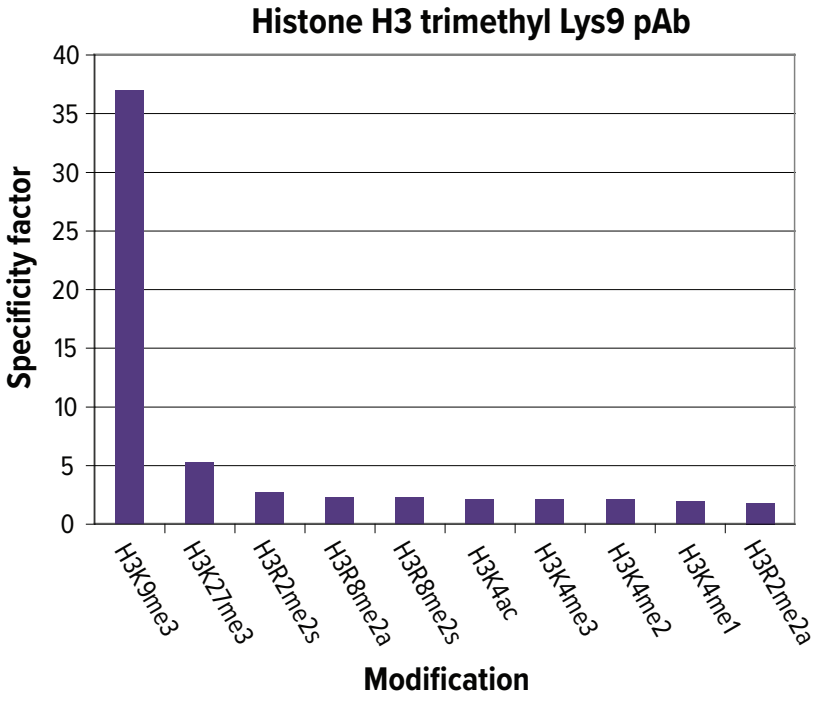


Image of ECL detection of Histone H3 trimethyl Lys9 (H3K9me3) pAb.

Active Motif's Histone H3 trimethyl Lys9 pAb (Cat. 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 Analyse 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 (Cat. No. 39161) has very little cross-reactivity with other histone modifications.

Kit Components and Storage

MODified™ Histone Peptide Arrays and the MODified™ Array Labeling Kit are for research use only. Not for use in diagnostic procedures. Please store each component at the temperature indicated in the table below. Kit and components are guaranteed for 6 months after receipt when stored properly.

Reagents	Quantity	Storage
Blocking Buffer AM2	100 ml	-20°C
10X Wash Buffer AM4	100 ml	4°C
AbFlex® Myc-Tag rAb	5 µl	-20°C
Anti-mouse HRP-conjugated secondary antibody	10 µl	4°C
Anti-rabbit HRP-conjugated secondary antibody	10 µl	4°C
ECL Reagent A	50 µl	4°C
ECL Reagent B	2 x 13 ml	4°C

Additional Materials Required

- MODified™ Histone Peptide Arrays (these are not included in the MODified™ Array Labeling kit)
- 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, only if using a sample antibody from a species other than mouse or rabbit
- Luminescent imaging system with CCD camera or X-ray film
- NUNC Well Rectangular Dishes (Cat. No. 267061) or suitable chamber for array incubation
- Optional: Active Motif's free Array Analyse Software program capable of analyzing MODified™ Histone Peptide Arrays (available for download at activemotif.com/MODified)

Array Specifications (not included in the MODified™ Array Labeling 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.

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 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 Buffer AM2. As a general guideline we suggest using monoclonal antibodies at a 1:2,000 dilution. For polyclonal antibodies, the amount recommended by the antibody provider for Western blot is usually sufficient.

Use a corresponding species specific secondary antibody conjugated to horseradish peroxidase (HRP). Dilute conjugated secondary antibody, as recommended for Western blot by the antibody provider, in 3 ml Blocking Buffer AM2 (1:2,500 is a suitable dilution for most secondary antibodies).

HRP-conjugated secondary antibodies

HRP-conjugated secondary anti-rabbit and anti-mouse antibodies are included in the kit. Sufficient antibody is provided for the detection of five arrays. Both the anti-rabbit and anti-mouse HRP-conjugated antibodies should be diluted 1:2,500 in Blocking Buffer AM2 (i.e. use 1.2 µl HRP-conjugated antibody per 3 ml Blocking Buffer AM2).

Control Peptides

The MODified™ Histone Peptide Array contains control spots (locations P20-P24), as referenced on the spreadsheet (activemotif.com/MODified). The control Myc-Tag peptide can be detected with the included Myc-Tag antibody as explained below. The control biotin peptide can be detected with either an anti-biotin antibody or a streptavidin-conjugated antibody (not included). The array also contains a no histone negative control peptide and two spots for background levels.

Note: Blocking Buffer AM2 may contain trace biotin molecules which can sequester the anti-biotin or streptavidin-conjugated 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 1 X Wash Buffer instead of using Blocking Buffer AM2.

Control Myc-Tag Antibody

The control Myc-Tag antibody is provided for the detection of the Myc-Tag peptide at location P21 on the MODified™ Histone Peptide Array. The Myc-Tag antibody can be combined directly with the sample antibody and applied to the array. (For sequential detection of control and sample antibodies, please refer to Appendix A).

The Myc-Tag antibody should be used at a 1:3,000 dilution. Add 1 µl of Myc-Tag antibody to 3 ml Blocking Buffer AM2 also containing the appropriate dilution of sample antibody.

For conjugated secondary antibody detection of the Myc-Tag control antibody, the anti-mouse HRP-conjugated secondary antibody should be diluted 1:2,500 in Blocking Buffer AM2 (i.e. use 1.2 µl HRP-conjugated antibody per 3 ml Blocking Buffer AM2).

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. 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.

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 Buffer AM2	**TOTAL REQUIRED**	**10 ml**	**50 ml**
	1X Wash Buffer	10X Wash Buffer AM4	9 ml	45 ml
		Distilled Water	81 ml	405 ml
		TOTAL REQUIRED	**90 ml**	**450 ml**
	ECL Reagent A	**TOTAL REQUIRED**	**1.5 µl**	**7.5 µl**
	ECL Reagent B	**TOTAL REQUIRED**	**5 ml**	**25 ml**
Antibody	Blocking Buffer AM2	**TOTAL REQUIRED**	**10 ml**	**50 ml**
	1X Wash Buffer	10X Wash Buffer AM4	7 ml	35 ml
		Distilled water	63 ml	315 ml
		TOTAL REQUIRED	**70 ml**	**350 ml**
	ECL Reagent A	**TOTAL REQUIRED**	**1.5 µl**	**7.5 µl**
	ECL Reagent B	**TOTAL REQUIRED**	**5 ml**	**25 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.

MODified™ Histone Peptide Array Labeling Protocol

Please read the entire protocol before use.

The following protocol is for screening antibody cross-reactivity or protein and enzyme interactions with histones and histone modifications using a chemiluminescent detection method. Prepare the Blocking Buffer and 1X Wash Buffer as described in the previous 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 1X Wash Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml 1X Wash Buffer.
6. 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.
7. Immerse one array in 3 ml Blocking Buffer AM2.
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 1X Wash Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml 1X Wash Buffer.
10. During the last wash step, prepare appropriate antibody dilutions.
11. Dilute the primary antibody in 3 ml Blocking Buffer AM2. See the dilution suggestions in the Buffer Preparation and Recommendations section on page 8.

Note: To detect the Myc-tag control peptide on the array, combine the included Myc-tag antibody at a 1:3,000 dilution with the diluted sample antibody in 3 ml Blocking Buffer AM2. 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 1X Wash Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml 1X Wash Buffer.
14. During the last wash step, prepare the secondary antibody dilution.

Note: To detect the Myc-tag control antibody, combine the included anti-mouse HRP-conjugated antibody at a 1:2,500 dilution with the diluted species specific secondary antibody for the sample antibody in 3 ml Blocking Buffer AM2.

15. Proceed to Step 4.

Step 2: Protein 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 3 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 three times for 5 minutes each on an orbital shaker using 5 ml 1X Wash 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 3 ml of protein binding buffer. Incubate with the blocked array for the desired time.
7. 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.
8. During the last wash step, prepare appropriate antibody dilutions.
9. Dilute the primary antibody in 3 ml Blocking Buffer AM2. See the dilution suggestions in the Buffer Preparation and Recommendations section on page 8.

Note: To detect the Myc-tag control peptide on the array, combine the included Myc-tag antibody at a 1:3,000 dilution with the diluted sample antibody in 3 ml Blocking Buffer AM2. 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 1X Wash Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml 1X Wash Buffer.
12. During the last wash step, prepare the secondary antibody dilution.

Note: To detect the Myc-tag control antibody, combine the included anti-mouse HRP-conjugated antibody at a 1:2,500 dilution with the diluted species specific secondary antibody for the sample in 3 ml Blocking Buffer AM2.

13. Proceed to Step 4.

Step 3: Antibody Studies

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 3 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 three times for 5 minutes each on an orbital shaker using 5 ml 1X Wash Buffer.
5. During the last wash step, prepare appropriate antibody dilutions.
6. Dilute the primary antibody in 3 ml Blocking Buffer AM2. See the dilution suggestions in the Buffer Preparation and Recommendations section on page 8.

Note: To detect the Myc-tag control peptide on the array, combine the included Myc-tag antibody at a 1:3,000 dilution with the diluted sample antibody in 3 ml Blocking Buffer AM2. 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 1X Wash Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml 1X Wash Buffer.
9. During the last wash step, prepare the secondary antibody dilution.

Note: To detect the Myc-tag control antibody, combine the included anti-mouse HRP-conjugated antibody at a 1:2,500 dilution with the diluted species specific secondary antibody for the sample in 3 ml Blocking Buffer AM2.

10. 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 1X Wash Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml 1X Wash Buffer.
3. During the last wash step, prepare the Detecting Solution as stated in the Buffer Preparation and Recommendations section on page 8.

Step 5: Detection

1. The Detecting Solution 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.

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 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.

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 Analyse manual, for use in Active Motif's Array Analyse Software. Programs such as Adobe Photoshop or the freeware program, GIMP2 (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 activemotif.com/MODified™.

2. Active Motif's free Array Analyse 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 Analyse software is designed for use on PCs only. Please download the Array Analyse manual for information about the installation and operation of the program. Both the manual and the Array Analyse Software can be downloaded from our website at activemotif.com/MODified.

Note: The Array Analysis Software designates the control as c-Myc. Please note that c-Myc and Myc-tag are used interchangeably. We use a Myc-tag peptide present in the array.

(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 Buffer and 1X Wash 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 Buffer AM2. 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 Buffer AM2. 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 Buffer AM2. 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 1X Wash Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml 1X Wash Buffer.
4. During the last wash step, prepare the Myc-tag control antibody dilution in 3 ml Blocking Buffer AM2. The Myc-tag antibody should be used at a 1:3,000 dilution.
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 1X Wash Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml 1X Wash Buffer.
7. During the last wash step, prepare the secondary antibody dilution in 3 ml Blocking Buffer AM2. Add the species specific HRP-conjugated secondary antibody needed for detection of the sample antibody (the kit includes anti-rabbit and anti-mouse HRP-conjugated antibodies). The control Myc-tag antibody requires the addition of a 1:2,500 dilution of anti-mouse HRP-conjugated antibody for the secondary antibody dilution. 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 1X Wash Buffer. Then wash three times for 5 minutes each on an orbital shaker using 5 ml 1X Wash Buffer.
10. During the last wash step, prepare the Detecting Solution as stated in the Buffer Preparation and Recommendations section on page 8. Approximately 1.5 ml of Detecting Solution 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 Buffer AM2	**TOTAL REQUIRED**	**18 ml**	**80 ml**
	1X Wash Buffer	10X Wash Buffer AM4	15 ml	65 ml
		Distilled Water	135 ml	585 ml
		TOTAL REQUIRED	**150 ml**	**650 ml**
	ECL Reagent A	**TOTAL REQUIRED**	**1.5 µl**	**7.5 µl**
	ECL Reagent B	**TOTAL REQUIRED**	**5 ml**	**25 ml**
Antibody	Blocking Buffer AM2	**TOTAL REQUIRED**	**18 ml**	**80 ml**
	1X Wash Buffer	10X Wash Buffer AM4	11 ml	55 ml
		Distilled water	99 ml	495 ml
		TOTAL REQUIRED	**110 ml**	**550 ml**
	ECL Reagent A	**TOTAL REQUIRED**	**1.5 µl**	**7.5 µl**
	ECL Reagent B	**TOTAL REQUIRED**	**5 ml**	**25 ml**

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, use the amount recommended by the antibody provider for Western blot detection. If these recommendations were followed and there is still high background, we suggest diluting the antibody 2 to 5-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.

Problem/Question	Possible Cause	Recommendation
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.

Technical Services

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

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	Direct:	760 431 1263
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