AbSurance™ Histone Antibody Specificity Arrays

Histone H3 (Catalog No. 16-667)
Histone H2A, H2B, H4 (Catalog No. 16-665)
Complete Core Histone Set (Catalog No. 16-668)

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

Current experimental approaches to elucidate mechanisms of epigenetic regulation, such as chromatin immunoprecipitation (ChIP) assays, are critically dependent on robust antibodies that can detect protein targets with a high degree of specificity. This is especially true when using antibodies directed against modified histones. Histones often undergo a number of post-translational modifications (PTM) at their N- and C-terminal tails. The most widely studied of these modifications are methylation, acetylation, and phosphorylation. Antibodies directed against modified histone residues should detect only one specific type of modification and discriminate between similarly modified residues (mono-, di-, or tri-adducts). The AbSurance™ Histone Antibody Specificity Arrays provide an easy and effective Western-blot–like approach to evaluate specificity and cross-reactivity of antibodies for histones H2A, H2B, H3, and H4, without the need for specialized imaging equipment or analysis software.

Kit Overview

The AbSurance™ Histone Antibody Specificity Arrays are comprised of high quality histone peptides (>95 % purity) spotted on Immobilon® P PVDF membranes in a 96-well format. The Histone H3 Array contains 100 ng and 10 ng quantities of 46 different H3 peptides: 8 acetylated, 27 methylated, 5 phosphorylated, and 6 unmodified. The Histone H2A, H2B, H4 Array contains 100 ng and 10 ng quantities of 43 peptides of H2A, H2B, and H4: 13 acetylated, 16 methylated, 6 phosphorylated, and 8 unmodified. Each Array is also spotted with 10 ng of normal IgG from rat, mouse, sheep, and rabbit, located at the bottom right corner of each Array, to serve as positive controls and location references. The Arrays are also cut at the upper left corner in the “A1” position as an additional location reference mark. Peptides are evenly distributed on the array to enable quantification of distinct spots, and accurate determination of cross-reactivity. The format of the AbSurance Histone Antibody Specificity Arrays enables a Western-blot–like detection approach using standard chemiluminescent reagents followed by image capture with either film or a CCD imaging system.

For Research Use Only; Not for use in diagnostic procedures

Kit Components

The AbSurance™ Histone Antibody Specificity Array is available in two formats: the Histone H3 Array (Cat. No. 16-667) consists of 46 different modified and unmodified H3 peptides, and the Histone H2A, H2B, H4 Array (Cat. No. 16-665) contains 43 different modified and unmodified peptides of histone H2A, H2B, and H4. These two arrays are available as a set, for a total of 89 different peptides (H2A, H2B, H3, H4), in the Complete Core Histone Set (Cat. No. 16-668).

Important: Peptides were spotted on each Array using an inert red dye that is visible on each membrane to demonstrate the location of distinct peptide spots. The intensity of the red spot **does not** indicate the quantity of peptide present at each spot location. Each peptide is spotted in 100 ng and 10 ng quantities (see location maps on page 3), and is not visible by direct inspection of the membrane.

Catalog No.	Description	Quantity
16-667	**AbSurance Histone H3 Antibody Specificity Array Store at 20-24°C**	
CS210990	AbSurance Histone H3 Antibody Specificity Array	1
16-665	**AbSurance Histone H2A, H2B, H4 Antibody Specificity Array Store at 20-24°C**	
CS210989	AbSurance Histone H2A, H2B, H4 Antibody Specificity Array	1
16-668	**AbSurance Complete Core Histone Antibody Specificity Array Store at 20-24°C**	
CS210990	AbSurance Histone H3 Antibody Specificity Array	1
CS210989	AbSurance Histone H2A, H2B, H4 Antibody Specificity Array	1

Additional Materials Required

- Primary Antibody
- Secondary Antibody
- Methanol
- TBST Solution (Tris-HCl (ph 7.4); 0.15 M NaCl, and 0.05% Tween® 20)
- Blocking Solution (5 % non-fat dried milk in TBST)
- Immobilon® Western Chemiluminescent HRP Substrate (Cat. No. WBKLS0100) or RapidStep™ ECL Reagent (Cat. No. 345818-100ML)
- Containers for the membrane (e.g. Nunc® dishes, Cat. No. 267060)
- Shaker platform
- Micropipettes and pipette tips
- Film or Imager (e.g. Bio-Rad ChemiDoc™ System)

Storage

The AbSurance Histone Antibody Specificity Array membranes (Cat. No. 16-665, 16-667, and 16-668) can be stored at 20 to 24°C (room temperature) for up to 6 months.

Location of Peptides on the AbSurance Histone Antibody Specificity Arrays

Histone H2A, H2B, H4 Array

	1	2	3	4	5	6	7	8	9	10	11	12	
A	H2A 1-19 unmod	H2A 1-19 S1P	H2A 1-19 K5ac	H2A 1-19 K9ac	H2A 1-19 K13ac	H2A 110-129 unmod	H2A 110-129 T120P	H2A.X 124-142 unmod	H2A.X 124-142 S139P	H2A.X 124-142 Y142P	H2B 1-19 unmod	H2B 1-19 K5ac	**100 ng**
B	H2A 1-19 unmod	H2A 1-19 S1P	H2A 1-19 K5ac	H2A 1-19 K9ac	H2A 1-19 K13ac	H2A 110-129 unmod	H2A 110-129 T120P	H2A.X 124-142 unmod	H2A.X 124-142 S139P	H2A.X 124-142 Y142P	H2B 1-19 unmod	H2B 1-19 K5ac	**10 ng**
C	H2B 1-19 K5me1	H2B 1-19 K12ac	H2B 1-19 S14P	H2B 1-19 K15ac	H2B 107-125 unmod	H2B 107-125 K120ac	H4 1-19 unmod	H4 1-19 S1P	H4 1-19 R3me1	H4 1-19 R3me2a	H4 1-19 R3me2s	H4 1-19 K5ac	**100 ng**
D	H2B 1-19 K5me1	H2B 1-19 K12ac	H2B 1-19 S14P	H2B 1-19 K15ac	H2B 107-125 unmod	H2B 107-125 K120ac	H4 1-19 unmod	H4 1-19 S1P	H4 1-19 R3me1	H4 1-19 R3me2a	H4 1-19 R3me2s	H4 1-19 K5ac	**10 ng**
E	H4 1-19 K8ac	H4 1-19 K12ac	H4 11-30 unmod	H4 11-30 K16ac	H4 11-30 R17me1	H4 11-30 R17me2a	H4 11-30 R17me2s	H4 11-30 R19me1	H4 11-30 R19me2a	H4 11-30 R19me2s	H4 11-30 K20ac	H4 11-30 K20me1	**100 ng**
F	H4 1-19 K8ac	H4 1-19 K12ac	H4 11-30 unmod	H4 11-30 K16ac	H4 11-30 R17me1	H4 11-30 R17me2a	H4 11-30 R17me2s	H4 11-30 R19me1	H4 11-30 R19me2a	H4 11-30 R19me2s	H4 11-30 K20ac	H4 11-30 K20me1	**10 ng**
G	H4 11-30 K20me2	H4 11-30 K20me3	H4 11-30 R23me1	H4 11-30 R23me2a	H4 11-30 R23me2s	H4 82-100 unmod	H4 82-100 K91ac	**100 ng**			Rat IgG	Sheep IgG	**10 ng**
H	H4 11-30 K20me2	H4 11-30 K20me3	H4 11-30 R23me1	H4 11-30 R23me2a	H4 11-30 R23me2s	H4 82-100 unmod	H4 82-100 K91ac	**10 ng**			Mouse IgG	Rabbit IgG	**10 ng**

Histone H3 Array

	1	2	3	4	5	6	7	8	9	10	11	12	
A	H3 1-19 unmod	H3 1-19 R2me1	H3 1-19 R2me2a	H3 1-19 R2me2s	H3 1-19 T3P	H3 1-19 K4ac	H3 1-19 K4me1	H3 1-19 K4me2	H3 1-19 K4me3	H3 1-19 R8me1	H3 1-19 R8me2a	H3 1-19 R8me2s	**100 ng**
B	H3 1-19 unmod	H3 1-19 R2me1	H3 1-19 R2me2a	H3 1-19 R2me2s	H3 1-19 T3P	H3 1-19 K4ac	H3 1-19 K4me1	H3 1-19 K4me2	H3 1-19 K4me3	H3 1-19 R8me1	H3 1-19 R8me2a	H3 1-19 R8me2s	**10 ng**
C	H3 1-19 K9ac	H3 1-19 K9me1	H3 1-19 K9me2	H3 1-19 K9me3	H3 1-19 S10P	H3 1-19 T11P	H3 7-26 unmod	H3 7-26 K14ac	H3 7-26 R17me1	H3 7-26 R17me2a	H3 7-26 R17me2s	H3 7-26 K18ac	**100 ng**
D	H3 1-19 K9ac	H3 1-19 K9me1	H3 1-19 K9me2	H3 1-19 K9me3	H3 1-19 S10P	H3 1-19 T11P	H3 7-26 unmod	H3 7-26 K14ac	H3 7-26 R17me1	H3 7-26 R17me2a	H3 7-26 R17me2s	H3 7-26 K18ac	**10 ng**
E	H3 16-34 unmod	H3 16-34 K23ac	H3 16-34 K27ac	H3 16-34 K27me1	H3 16-34 K27me2	H3 16-34 K27me3	H3 16-34 R26me1	H3 16-34 R26me2a	H3 16-34 R26me2s	H3 16-34 S28P	H3 26-44 unmod	H3 26-44 K36ac	**100 ng**
F	H3 16-34 unmod	H3 16-34 K23ac	H3 16-34 K27ac	H3 16-34 K27me1	H3 16-34 K27me2	H3 16-34 K27me3	H3 16-34 R26me1	H3 16-34 R26me2a	H3 16-34 R26me2s	H3 16-34 S28P	H3 26-44 unmod	H3 26-44 K36ac	**10 ng**
G	H3 26-44 K36me1	H3 26-44 K36me2	H3 26-44 K36me3	H3 26-44 Y41P	H3 47-65 unmod	H3 47-65 K56ac	H3 71-89 unmod	H3 71-89 K79me1	H3 71-89 K79me2	H3 71-89 K79me3	Rat IgG **10 ng**	Sheep IgG **10 ng**	**100 ng**
H	H3 26-44 K36me1	H3 26-44 K36me2	H3 26-44 K36me3	H3 26-44 Y41P	H3 47-65 unmod	H3 47-65 K56ac	H3 71-89 unmod	H3 71-89 K79me1	H3 71-89 K79me2	H3 71-89 K79me3	Mouse IgG **10 ng**	Rabbit IgG **10 ng**	**10 ng**

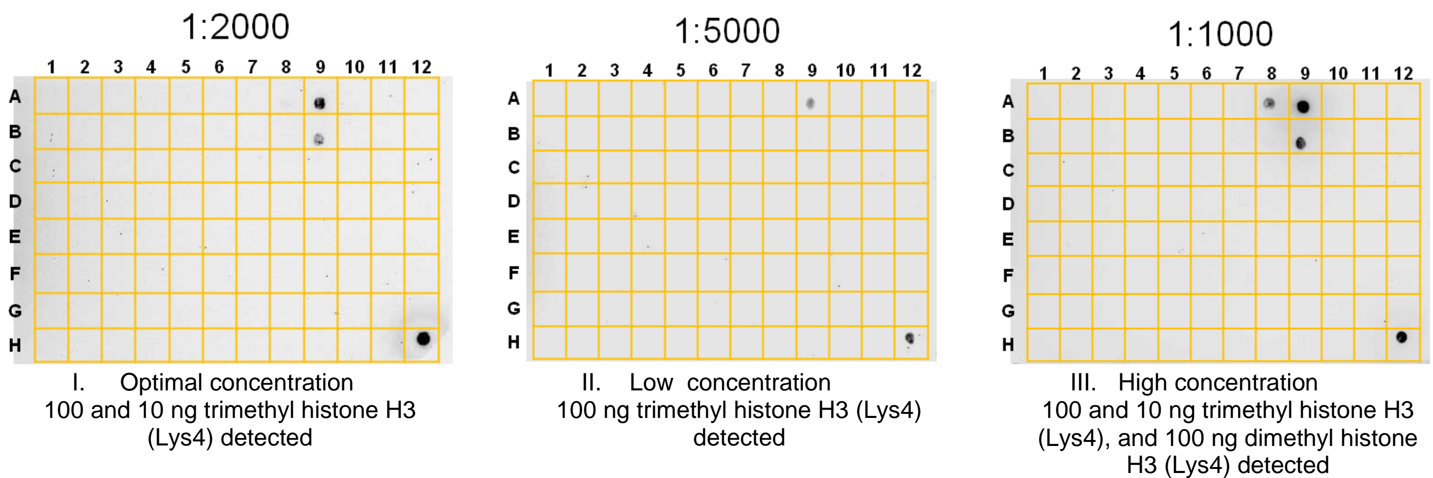
Experimental Considerations

1. Dilution of Antibodies

a) Primary Antibodies

Follow the vendor's recommendations for dot blot assays when diluting the primary antibody. If there is no information for dot blot assays, optimization of the antibody dilution is strongly recommended. If the antibody concentration is too high, there could be potential interactions that would not be observed in other types of assays. With an optimized dilution, both 100 ng and 10 ng spots of the target peptides on the AbSurance Histone Antibody Specificity Arrays can be detected, as shown in Figure 3 (I). Low concentrations of antibodies may result in detection of only the 100 ng spots (see Figure 3 (II)), and a high concentration of antibody may detect weak cross-reactivity with peptides of similar modification, as shown in Figure 3 (III).

Figure 3: Optimization of dilution for anti-trimethyl histone H3 (Lys4)
The Histone H3 Array was probed with varying concentrations of anti-trimethyl histone H3 (Lys) and peptides were visualized with a donkey anti-rabbit IgG, peroxidase conjugated, H+L, and a chemiluminescent system.



I. Optimal concentration
100 and 10 ng trimethyl histone H3 (Lys4) detected

II. Low concentration
100 ng trimethyl histone H3 (Lys4) detected

III. High concentration
100 and 10 ng trimethyl histone H3 (Lys4), and 100 ng dimethyl histone H3 (Lys4) detected

b) Secondary Antibodies

Use corresponding species-specific HRP-conjugated secondary antibodies at dilutions recommended for Western blot. We recommend goat anti-mouse IgG, peroxidase conjugated, H+L (Cat. No. AP124P) for mouse antibodies, and donkey anti-rabbit IgG, peroxidase conjugated, H+L (Cat. No. AP182P) for rabbit antibodies.

2. Detection Methods

Chemiluminescent detection with film or a CCD imaging system is recommended. Colorimetric detection methods have lower sensitivity and are not recommended.

Spot intensity after suggested exposure times may differ depending on whether film or CCD imaging was used. Typically, CCD imagers produce weaker signals than film after the same exposure time; a 10 minute exposure to the CCD imager is roughly equivalent to a 1 minute exposure to film (Figure 3 (I to III)). When using film we recommend the RapidStep™ ECL Reagent (Cat. No. 345818-100ML); and when using the CCD imager, we recommend highly sensitive detection reagents such as the Immobilon® Western Chemiluminescent HRP Substrate (Cat. Nos. WBKLS0050, WBKLS0100).

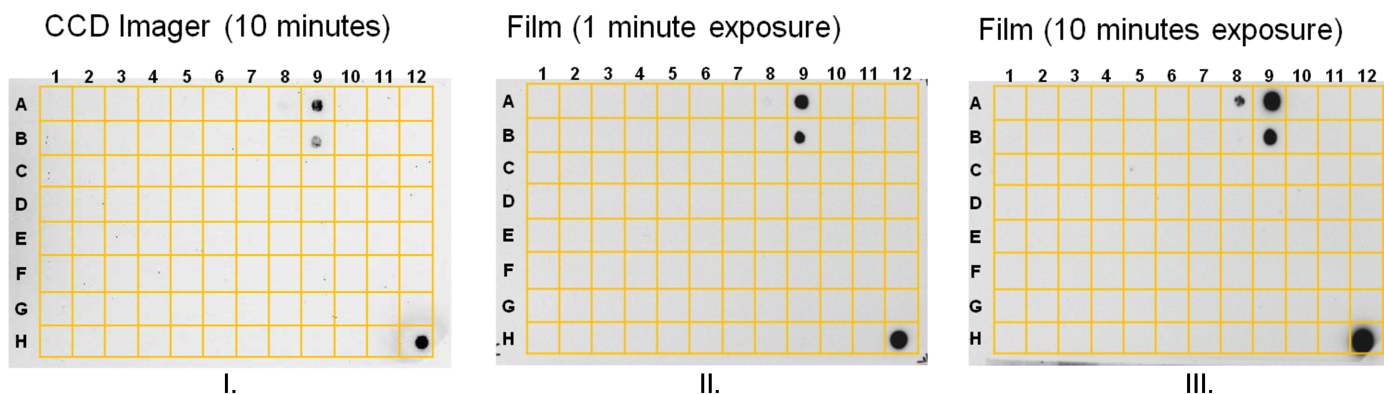


Figure 3: Comparison of detection with CCD imager and film
The Histone H3 Array was probed with anti-trimethyl histone H3 (Lys4) (1:2000 dilution; Cat. No. 05-745R) and peptides were visualized with an HRP-conjugated secondary antibody.

I. Peptides were visualized with Immobilon® Western Chemiluminescent HRP Substrate and the image was developed using CCD imager (Bio-Rad ChemiDoc™ system) for 10 minutes.
II. Peptides were visualized with RapidStep™ ECL reagent and exposed to film for 1 minute.
III. Peptides were visualized with RapidStep™ ECL reagent and exposed to film for 10 minutes.

Protocol

Important: To avoid contamination with proteins from your finger tips, wear gloves at all times when handling the AbSurance Histone Antibody Specificity Array membranes. Handle only the edges of the membranes with forceps to prevent damage to spot locations and potential inconsistencies in spot intensity after detection.

Optional Spot Alignment Tool: An alignment grid of the Histone Array that is identical in size and placement of the spots to the membrane is provided on page 7. If desired, create a transparent copy of this grid to assist with locating and identifying reactive spots on the membrane.

A. Antibody Screening

1. Rehydrate the membrane by soaking in 20 mL of methanol (ensure that the membrane is completely immersed). Rinse the membrane in 20 mL of TBST solution.
2. Block the membrane with 20 mL of blocking solution (5% milk in TBST) for 1 hour at RT with gentle shaking (approximately 60 rpm).
3. Add the appropriate amount of primary antibody (see “Experimental Considerations” section) to the blocking solution to obtain the desired concentration.
4. Incubate the membrane for 1 hour at RT or at 4℃ overnight with gentle shaking (approximately 60 rpm).
5. Discard primary antibody solution; wash excess primary antibody from the membrane by adding 20 mL of TBST and incubating at RT for 5 mins on the shaker platform (approximately 60 rpm).
6. Repeat wash outlined in Step 5 two (2) times.
7. Add 20 mL of the pre-diluted secondary antibody and incubate for 1 hour at RT on the shaker platform (approximately 60 rpm).
8. Discard secondary antibody solution; wash excess secondary antibody from membrane by adding 20 mL of TBST and incubating at RT for 5 mins on the shaker (approximately 60 rpm).
9. Repeat wash outlined in Step 8 three (3) times. During the last incubation, prepare the detection solution according to the manufacturer's instructions.

B. Detection and Analysis using Immobilon Western Chemiluminescent HRP Substate

Note: For other detection systems, follow the vendor’s protocol and use the information below only as a guide.

<u>Chemiluminescent detection with CCD camera imager</u>

1. Remove Immobilon® Western Chemiluminescent HRP Substrate from the refrigerator and allow it to reach RT before use (approximately 10 minutes).
2. To prepare working HRP substrate for one membrane, mix 2 mL of Luminol reagent and 2 mL of peroxide solution in a 15 or 50 mL conical tube. (This should be protected from light.)
3. Remove the AbSurance Histone Antibody Specificity Array membrane from the last wash solution and place it on a flat surface, protein side up. Do not allow the membrane to dry.
4. Pour the mixed ECL substrate onto the membrane and incubate for 1-2 minutes.
5. Capture images of multiple exposure times (1 to 15 mins) with a CCD Imager.
6. Overlay the alignment grid provided on page 7, using the cut A1 position, and the positive control IgG spot at the bottom right of the membrane, as reference marks.

Chemiluminescent detection with Film

1. Remove the RapidStep™ ECL reagent from the refrigerator and allow it to reach RT before use (approximately 10 minutes).
2. Remove the AbSurance Histone Antibody Specificity Array membrane from the last wash solution and place it on a flat surface, protein side up. Do not allow the membrane to dry.
3. Spray the RapidStep™ ECL reagent on the membrane, with enough reagent to cover the entire surface of the membrane. Incubate for 1-2 minutes. Remove excess ECL and wrap the membrane in Saran wrap, avoiding air bubbles.
4. Place the wrapped membrane in a film cassette and expose to film.
5. Develop the images in a dark room for the desired exposure time.
6. Overlay the alignment grid provided on page 7, using the cut A1 position, and the positive control IgG spot at the bottom right of the membrane, as reference marks.

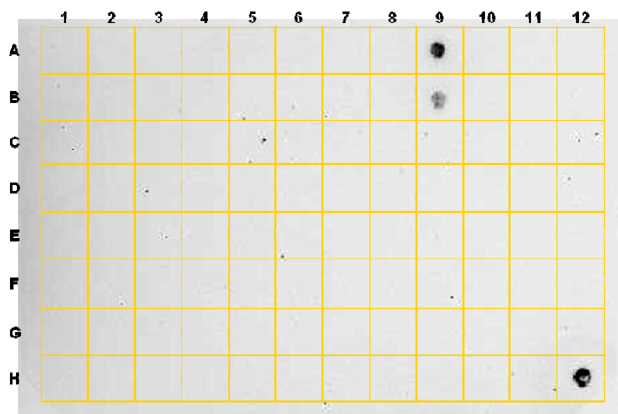
Spot Alignment Grid for Location and Identification of Reactive Peptide Spots

	1	2	3	4	5	6	7	8	9	10	11	12	
A													**100ng**
B													**10ng**
C													**100ng**
D													**10ng**
E													**100ng**
F													**10ng**
G													**100ng**
H													**10ng**

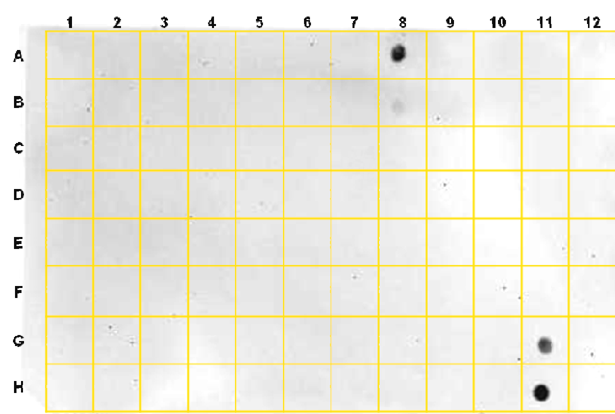
Actual Size Grid: Copy this grid onto a transparent sheet and align the cut edge of your AbSurance Histone Antibody Specificity Array membrane with position A1 on this grid, and use the appropriate location array map on page 3 to correctly identify reactive spots on your membrane.

Representative Screening Assays: Histone H3 Array

I. Trimethyl Histone H3 (Lys4)



II. Dimethyl Histone H3 (Lys4)



III. Location of reactivity with histone H3 antibodies and control IgGs tested

	1	2	3	4	5	6	7	8	9	10	11	12	
A	H3 1-19 unmod	H3 1-19 R2me1	H3 1-19 R2me2a	H3 1-19 R2me2s	H3 1-19 T3P	H3 1-19 K4ac	H3 1-19 K4me1	H3 1-19 K4me2	H3 1-19 K4me3	H3 1-19 R8me1	H3 1-19 R8me2a	H3 1-19 R8me2s	**100 ng**
B	H3 1-19 unmod	H3 1-19 R2me1	H3 1-19 R2me2a	H3 1-19 R2me2s	H3 1-19 T3P	H3 1-19 K4ac	H3 1-19 K4me1	H3 1-19 K4me2	H3 1-19 K4me3	H3 1-19 R8me1	H3 1-19 R8me2a	H3 1-19 R8me2s	**10 ng**
C	H3 1-19 K9ac	H3 1-19 K9me1	H3 1-19 K9me2	H3 1-19 K9me3	H3 1-19 S10P	H3 1-19 T11P	H3 7-26 unmod	H3 7-26 K14ac	H3 7-26 R17me1	H3 7-26 R17me2a	H3 7-26 R17me2s	H3 7-26 K18ac	**100 ng**
D	H3 1-19 K9ac	H3 1-19 K9me1	H3 1-19 K9me2	H3 1-19 K9me3	H3 1-19 S10P	H3 1-19 T11P	H3 7-26 unmod	H3 7-26 K14ac	H3 7-26 R17me1	H3 7-26 R17me2a	H3 7-26 R17me2s	H3 7-26 K18ac	**10 ng**
E	H3 16-34 unmod	H3 16-34 K23ac	H3 16-34 K27ac	H3 16-34 K27me1	H3 16-34 K27me2	H3 16-34 K27me3	H3 16-34 R26me1	H3 16-34 R26me2a	H3 16-34 R26me2s	H3 16-34 S28P	H3 26-44 unmod	H3 26-44 K36ac	**100 ng**
F	H3 16-34 unmod	H3 16-34 K23ac	H3 16-34 K27ac	H3 16-34 K27me1	H3 16-34 K27me2	H3 16-34 K27me3	H3 16-34 R26me1	H3 16-34 R26me2a	H3 16-34 R26me2s	H3 16-34 S28P	H3 26-44 unmod	H3 26-44 K36ac	**10 ng**
G	H3 26-44 K36me1	H3 26-44 K36me2	H3 26-44 K36me3	H3 26-44 Y41P	H3 47-65 unmod	H3 47-65 K56ac	H3 71-89 unmod	H3 71-89 K79me1	H3 71-89 K79me2	H3 71-89 K79me3	Rat IgG **10 ng**	Sheep IgG **10 ng**	**100 ng**
H	H3 26-44 K36me1	H3 26-44 K36me2	H3 26-44 K36me3	H3 26-44 Y41P	H3 47-65 unmod	H3 47-65 K56ac	H3 71-89 unmod	H3 71-89 K79me1	H3 71-89 K79me2	H3 71-89 K79me3	Mouse IgG **10 ng**	Rabbit IgG **10 ng**	**10 ng**

Figure 1: Specificity screening of histone H3 antibodies

I. The Histone H3 Array was probed with anti-trimethyl histone H3 (Lys4) (Cat.No. 05-745R; 1µg/mL). Peptides and control IgG were visualized using a donkey anti-rabbit IgG, peroxidase conjugated, H+L (Cat. No. AP182P) and a chemiluminescent detection system.

II. The Histone H3 Array was probed with anti-dimethyl histone H3 (Lys4) (Cat. No. 05-1338, 1µg/mL). Peptides were visualized using a goat anti-mouse IgG peroxidase conjugated, H+L (Cat. No. AP124P) and a chemiluminescence detection system.

III. Peptide map showing reactive peptide spots on Figure 1(I) (RED) and Figure 1(II) (BLUE). Respective species-specific control IgG, which reacted with the secondary antibody used, is shown in a lighter shade of each color.

Representative Screening Assays: Histone H2A, H2B, H4 Array

I. Acetyl Histone H4 (Lys12)

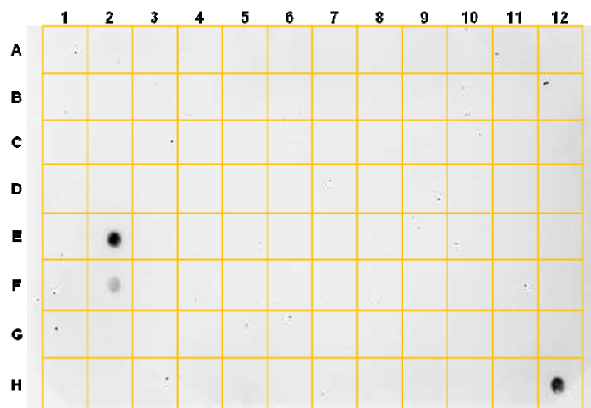


Figure 2: Specificity screening of histone H4 antibody

I. The Histone H2A, H2B, H4 Array was probed with anti-acetyl histone H4 (Lys12) antibody (1:2000 dilution, Cat. #04-119). Peptides were visualized using a donkey anti-rabbit IgG, peroxidase conjugated, H+L (Cat. #AP182P) secondary antibody and a chemiluminescence detection system

II. Peptide map showing location of reactive peptide spots on Figure 2(I) (Yellow). Control rabbit IgG is shown in lighter shade of yellow.

II. Location of reactivity with the H4 antibody and control IgG tested

	1	2	3	4	5	6	7	8	9	10	11	12	
A	H2A 1-19 unmod	H2A 1-19 S1P	H2A 1-19 K5ac	H2A 1-19 K9ac	H2A 1-19 K13ac	H2A 110-129 unmod	H2A 110-129 T120P	H2A.X 124-142 unmod	H2A.X 124-142 S139P	H2A.X 124-142 Y142P	H2B 1-19 unmod	H2B 1-19 K5ac	**100 ng**
B	H2A 1-19 unmod	H2A 1-19 S1P	H2A 1-19 K5ac	H2A 1-19 K9ac	H2A 1-19 K13ac	H2A 110-129 unmod	H2A 110-129 T120P	H2A.X 124-142 unmod	H2A.X 124-142 S139P	H2A.X 124-142 Y142P	H2B 1-19 unmod	H2B 1-19 K5ac	**10 ng**
C	H2B 1-19 K5me1	H2B 1-19 K12ac	H2B 1-19 S14P	H2B 1-19 K15ac	H2B 107-125 unmod	H2B 107-125 K120ac	H4 1-19 unmod	H4 1-19 S1P	H4 1-19 R3me1	H4 1-19 R3me2a	H4 1-19 R3me2s	H4 1-19 K5ac	**100 ng**
D	H2B 1-19 K5me1	H2B 1-19 K12ac	H2B 1-19 S14P	H2B 1-19 K15ac	H2B 107-125 unmod	H2B 107-125 K120ac	H4 1-19 unmod	H4 1-19 S1P	H4 1-19 R3me1	H4 1-19 R3me2a	H4 1-19 R3me2s	H4 1-19 K5ac	**10 ng**
E	H4 1-19 K8ac	H4 1-19 K12ac	H4 11-30 unmod	H4 11-30 K16ac	H4 11-30 R17me1	H4 11-30 R17me2a	H4 11-30 R17me2s	H4 11-30 R19me1	H4 11-30 R19me2a	H4 11-30 R19me2s	H4 11-30 K20ac	H4 11-30 K20me1	**100 ng**
F	H4 1-19 K8ac	H4 1-19 K12ac	H4 11-30 unmod	H4 11-30 K16ac	H4 11-30 R17me1	H4 11-30 R17me2a	H4 11-30 R17me2s	H4 11-30 R19me1	H4 11-30 R19me2a	H4 11-30 R19me2s	H4 11-30 K20ac	H4 11-30 K20me1	**10 ng**
G	H4 11-30 K20me2	H4 11-30 K20me3	H4 11-30 R23me1	H4 11-30 R23me2a	H4 11-30 R23me2s	H4 82-100 unmod	H4 82-100 K91ac	**100 ng**			Rat IgG	Sheep IgG	**10 ng**
H	H4 11-30 K20me2	H4 11-30 K20me3	H4 11-30 R23me1	H4 11-30 R23me2a	H4 11-30 R23me2s	H4 82-100 unmod	H4 82-100 K91ac	**10 ng**			Mouse IgG	Rabbit IgG	**10 ng**

Troubleshooting Guide

Step	Experimental Suggestions
Low signal or no signal from peptide spots after detection	The primary antibody may be too dilute. In general, use antibody concentrations recommended for dot blot assays, and if needed, proceed to optimize the antibody for this assay (see Experimental Considerations section for suggested starting points).
	The primary antibody may have lost activity. Multiple freeze-thaw cycles and bacterial contamination can change the activity of an antibody. Increase the dilution of the antibody, and use new containers when possible to avoid cross-contamination from other assays.
	The blocking reagent may have an affinity for the target and may bind to the peptide and prevent its detection. Reduce the concentration of the blocking agent or the blocking time, or try a different blocking reagent.
Low signal or no signal from neither peptide spots nor control IgG spots	Check that the ECL substrate has not expired and is still working properly. Adjust the settings of your CCD imager or increase the exposure time.
Unexpected High cross-reactivity or background signals	The concentration of the primary or secondary antibody may be too high (see Experimental Considerations). Adjust the concentration of the primary or secondary antibody, or both. Use only high quality antibodies. If high cross reactivity is observed even after optimizing antibody dilution, it is possible that the antibody cross reacts with other sites.
	Ensure that buffers are freshly prepared and free of contaminants.
	Increase the number of washes or the duration of washes, or both.
	Drain excess detection reagents from the membrane before exposing it, and reduce the exposure time, if necessary.
Re-probing Membranes	Stripping and re-probing of the AbSurance Histone Antibody Specificity Array membranes is not recommended. To avoid potential loss of sensitivity and misleading results, a new membrane should be used for each experiment.
Signal not uniform	Ensure that there are no air bubbles on the membrane during the ECL detection step.
	Use sufficient quantities of primary and secondary antibody solutions to ensure that the membrane is completely immersed in these solutions during the incubation period.
	It is important that the membrane is rehydrated before the experiment. Failure to do so may create inconsistencies in peptide spots.
	Fingerprints and marks from forceps may contribute to inconsistent signals. Wear gloves during the assay, and handle only the edges of the membrane when using forceps.

Kits and Accessories for Chromatin Immunoprecipitation

Magnetic ChIP Products	Catalog No.
Magna ChIP™ A Kit	17-610
Magna ChIP™ G Kit	17-611
EZ-Magna ChIP™ A Kit	17-408
EZ-Magna ChIP™ G Kit	17-409
Magna ChIP™ A/G Kit	17-10085
EZ-Magna ChIP™ A/G Kit	17-10086
Magna ChIP™ HT96 Kit	17-10077
EZ-Magna ChIP™ HT96 Kit	17-10078
Magna ChIP-Seq™ Chromatin Immunoprecipitation and Next Generation Sequencing Library Preparation Kit	17-1010
Magna ChIP^{2}™ Universal Chromatin Immunoprecipitation DNA Microarray Kit	17-1000
Magna ChIP^{2}™ Universal Chromatin Immunoprecipitation DNA Microarray Quad Kit	17-1004
Magna ChIP™ G Tissue Kit	17-20000
Magna ChIP™ Protein A+G Magnetic Beads	16-663
Magna ChIP™ Protein G Magnetic Beads	16-662
Magna ChIP™ Protein A Magnetic Beads	16-661
Magna GrIP™ Rack (8-well)	20-400
PureProteome® Magnetic Stand	LSKMAGS08
Agarose ChIP Products	**Catalog No.**
ChIP Assay Kit	17-295
EZ-ChIP™ Kit	17-371

Antibodies for Chromatin Immunoprecipitation

ChIPAb+ Validated Antibodies; with control IgG and qPCR primers	Catalog No.
ChIPAb+™ Histone H2A.Z	17-10048
ChIPAb+™ Histone H2B	17-10054
ChIPAb+™ Histone H3 (C-term)	17-10046
ChIPAb+™ Histone H3 (Unmod Lys4)	17-675
ChIPAb+™ Acetyl Histone H3	17-615
ChIPAb+™ Acetyl-Histone H3 (Lys4)	17-10050
ChIPAb+™ Acetyl-Histone H3 (Lys9)	17-658
ChIPAb+™ Acetyl-Histone H3 (Lys14)	17-10051
ChIPAb+™ Monomethyl Histone H3 (Lys27)	17-643
ChIPAb+™ Dimethyl-Histone H3 (Lys4)	17-677
ChIPAb+™ Dimethyl-Histone H3 (Lys9)	17-648
ChIPAb+™ Trimethyl-Histone H3 (Lys4)	17-614
ChIPAb+™ Trimethyl-Histone H3 (Lys4)	17-678
ChIPAb+™ Trimethyl-Histone H3 (Lys9)	17-625
ChIPAb+™ Trimethyl-Histone H3 (Lys27)	17-622
ChIPAb+™ Trimethyl-Histone H3 (Lys36)	17-10032
ChIPAb+™ Trimethyl-Histone H3 (Lys79)	17-10130
ChIPAb+™ Phospho-Histone H3 (Ser10)	17-685
ChIPAb+™ Acetyl Histone H4	17-630
ChIPAb+™ Acetyl-Histone H4 (Lys5)	17-10045
ChIPAb+™ CREB	17-600
ChIPAb+™ CTCF	17-10044
ChIPAb+™ EED	17-663
ChIPAb+™ EED (Rabbit Polyclonal)	17-10034
ChIPAb+™ ERα	17-603
ChIPAb+™ EZH2, clone AC22	17-662
ChIPAb+™ HDAC1	17-608
ChIPAb+™ p53	17-613
ChIPAb+™ Phospho-CREB (Ser133)	17-10131
ChIPAb+™ REST	17-641
ChIPAb+™ RNA Polymerase II	17-620
ChIPAb+™ SMRT	17-10057
ChIPAb+™ Sox-2, clone 6F1.2	17-656
ChIPAb+™ Sp1	17-601
ChIPAb+™ SUZ12	17-661
ChIPAb+™ TATA Binding Protein (TBP)	17-10098

References

Egelhofer, T.A.., *et al*. (2011). *Nat Struct Mol Biol.* 18(1):91-93.

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