Version 2 Last updated 12 April 2018

# ab233494 Histone H3 Peptide Array Kit (96-well plate-based)

For the measurement of the selectivity and specificity of antibodies against 46 different histone modifications and the identification of histone modifying enzyme substrates.

This product is for research use only and is not intended for diagnostic use.

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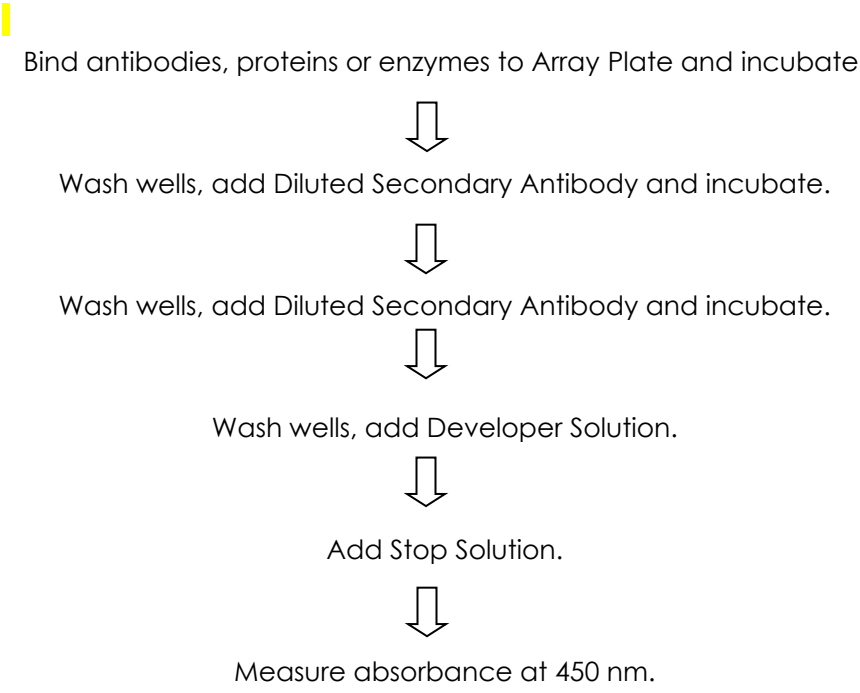
# 1. Overview

The Histone H3 Peptide Array ELISA Kit (96-well plate-based) (ab233494) is designed to rapidly examine the selectivity and specificity of antibodies against 46 different histone modifications and is also suitable for identifying substrates of histone modifying enzymes and analyzing specificity of histone binding proteins.

In an assay with this kit, the Histone H3 proteins (modified at specific sites) that are tightly arrayed on the wells are incubated with input materials such as antibodies, proteins or enzymes. After incubation, the input materials are detected through an ELISA reaction system, the binding intensity is proportional to the intensity of the absorbance.



## 2. Materials Supplied and Storage

Store kit at 4°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

| Item | Quantity (96 Assays) | Storage temperature (before prep) |
|---|---|---|
| 10X Wash Buffer | 28 ml | 4°C |
| Developer Solution | 12 ml | 4°C |
| Stop Solution | 10 ml | RT |
| Array Plate (96-well) | 1 | 4°C |
| Adhesive Covering Film | 1 | RT |

## 3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Adjustable pipette or multiple-channel pipette
- Multiple-channel pipette reservoirs
- Aerosol resistant pipette tips
- Microplate reader capable of reading absorbance at 450 nm
- 1.5 ml microcentrifuge tubes
- Incubator for 37°C incubation
- Distilled water
- Primary antibodies, proteins and/or enzymes of interest
- Secondary antibody conjugated with HRP
- Parafilm M or aluminum foil

## 4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

# 5. Reagent Preparation

- Briefly centrifuge small vials at low speed prior to opening.
- If Wash Buffer contains salt precipitates, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.

## 5.1 10X Wash Buffer

Prepare diluted 1X Wash Buffer by adding 26 ml of 10X Wash Buffer to 234 ml of distilled water and adjust pH to 7.2-7.5.

**ΔNote:** Diluted 1X Wash Buffer can now be stored at 4°C for up to six months.

## 5.2 Developer Solution

Ready to use as supplied.

**ΔNote**: Check if a blue color is present in Developer Solution, which would indicate contamination of the solution and should not be used. To avoid contamination, transfer the amount of Developer Solution required into a secondary container (tube or vial) before adding Developer Solution into the assay wells.

## 5.3 Stop Solution

Ready to use as supplied.

## 5.4 Array Plate (96-well)

Ready to use as supplied.

## 5.5 USER SUPPLIED REAGENT: Diluted Secondary Antibody (HRP-conjugated)

Dilute Secondary Antibody with diluted 1X Wash Buffer to 0.4 µg/ml (i.e., add 1 µl of primary antibody at concentration of 1 mg/ml to 2500 µl of 1X diluted Wash Buffer). 50 µl of Diluted Primary Antibody will be required for each assay well. The Secondary Antibody should be specific against the Primary Antibody (see Section 6, below).

**ΔNote:** Keep each of the diluted solutions except diluted 1X Wash Buffer on ice until use. Any remaining diluted solutions other than Diluted 1X Wash Buffer should be discarded if not used within the same day.

# 6. Sample Preparation

## General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.

### 6.1 Sample Antibodies:

To investigate the specificity of user-supplied anti-histone antibodies.
Dilute Primary Antibody with diluted 1X Wash Buffer to 1 µg/ml (i.e., add 1 µl of primary antibody at the concentration of 1 mg/ml to 1000 µl of Diluted WB). 50 µl of this Diluted Primary Antibody will be required for each assay well.

### 6.2 Sample Proteins:

To investigate the binding of user-supplied protein(s) of interest to histone proteins.
Prepare 5 ml of Sample Protein solution by diluting the sample protein with appropriate protein binding buffer to the desired concentration. Make sure the necessary co-factors are included in the buffer.
**ΔNote:** Use the appropriate primary antibody and species-specific secondary antibody accordingly. Prepare Diluted Primary Antibody and Diluted Secondary Antibody as directed (see Sections 6.1 and 5.5, respectively).

### 6.3 Sample Enzymes:

To investigate the action of user-supplied histone-modifying enzymes.
Prepare 5 ml of sample enzyme solution by diluting the enzyme with appropriate enzyme assay buffer to the desired concentration. Make sure the co-factors such as methyl donor-Adomet (for histone methyltransferase) are included in the assay buffer.
**ΔNote:** Use the appropriate primary antibody and species-specific secondary antibody accordingly. Prepare Diluted Primary Antibody and Diluted Secondary Antibody as directed (see Sections 6.1 and 5.5, respectively).

**ΔNote:** Keep each of the diluted sample solutions on ice until use. Any remaining diluted solutions should be discarded if not used within the same day.

# 7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

## 7.1 For Antibody Analysis:

1. Add 50 µl of Diluted Primary Antibody (see Step 5.4) to each well of the Array Plate.
2. Tightly cover the plate with Adhesive Covering Film to avoid evaporation and incubate at 37°C for 60 minutes.
3. Remove the reaction solution from each well. Wash each well three times with 150 µl of diluted 1X Wash Buffer each time.
4. Add 50 µl of Diluted Secondary Antibody (see Step 5.5) to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 30 minutes.
5. Remove the Diluted Secondary Antibody from each well.
6. Wash each well four times with 150 µl of diluted 1X Wash Buffer each time.

   **ΔNote:** Ensure any residual 1X Wash Buffer in the wells is removed as much as possible at each wash step.
7. Add 100 µl of Developer Solution to each well and incubate at room temperature for 1 to 10 minutes away from light. Begin monitoring color change in the sample wells and control wells. The Developer Solution will turn blue if the sample antibody binds to the histone peptides.
8. Add 100 µl of Stop Solution to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding Stop Solution and the absorbance should be read on a microplate reader within 2 to 10 minutes at 450 nm with an optional reference wavelength of 655 nm.

   **ΔNote**: Most microplate readers have the capability to carry out dual wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice, once at 450 nm

and once at 655 nm. Then, manually subtract the 655 nm ODs from 450 nm ODs.

**ΔNote:** If the strip-well microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.

## 7.2 For Protein Histone Binding Analysis:

1. Add 50 µl of diluted Sample Protein solution into each well of the Array Plate.
2. Tightly cover the plate with Adhesive Covering Film to avoid evaporation and incubate at an appropriate temperature for desired time.
3. Remove the reaction solution from each well. Wash each well three times with 150 µl of diluted 1X Wash Buffer each time.
4. Add 50 µl of Diluted Primary Antibody (see Section 6.1) to each well of Array Plate.

   **ΔNote:** The Diluted Primary Antibody should be specific for the Sample Protein(s).
5. Tightly cover the plate with Adhesive Covering Film to avoid evaporation and incubate at 37°C for 60 minutes.
6. Remove the reaction solution from each well. Wash each well three times with 150 µl of diluted 1X Wash Buffer each time.
7. Add 50 µl of the Diluted Secondary Antibody (see Section 5.5) to each well, then cover with Parafilm M or aluminium foil and incubate at room temperature for 30 minutes.

   **ΔNote:** The secondary antibody should be specific against the Diluted Primary Antibody.
8. Remove the Diluted Secondary Antibody from each well.
9. Wash each well four times with 150 µl of diluted 1X Wash Buffer each time.

   **ΔNote:** Ensure any residual 1X Wash Buffer in the wells is removed as much as possible at each wash step.
10. Add 100 µl of Developer Solution to each well and incubate at room temperature for 1 to 10 minutes away from light. Begin monitoring color change in the sample wells and

control wells. The Developer Solution solution will turn blue in the presence of sufficient histone-Sample Protein binding.

11. Add 100 µl of Stop Solution to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding Stop Solution and the absorbance should be read on a microplate reader within 2 to 10 minutes at 450 nm with an optional reference wavelength of 655 nm.

## 7.3 For Histone-Modifying Enzyme Interaction Analysis:

1. Add 50 µl of diluted Sample Enzyme solution into each well of Array Plate.
2. Tightly cover the plate with Adhesive Covering Film to avoid evaporation and incubate at an appropriate temperature for desired time.
3. Remove the reaction solution from each well. Wash each well three times with 150 µl of diluted 1X Wash Buffer each time.
4. Add 50 µl of Diluted Primary Antibody prepared (see Section 6.1) to each well of Array Plate.

   **ΔNote**: The primary antibody should be specific for the sample enzymes.
5. Tightly cover the plate with Adhesive Covering Film to avoid evaporation and incubate at 37°C for 60 minutes.
6. Remove the reaction solution from each well. Wash each well three times with 150 µl of diluted 1X Wash Buffer each time.
7. Add 50 µl of the Diluted Secondary Antibody (see Section 5.5) to each well, then cover with Parafilm M or aluminium foil and incubate at room temperature for 30 minutes.

   **ΔNote:** The secondary antibody should be specific against the Diluted Primary Antibody.
8. Remove the Diluted Secondary Antibody from each well.
9. Wash each well four times with 150 µl of diluted 1X Wash Buffer each time.

**ΔNote:** Ensure any residual 1X Wash Buffer in the wells is removed as much as possible at each wash step.

10. Add 100 µl of Developer Solution to each well and incubate at room temperature for 1 to 10 minutes away from light. Begin monitoring color change in the sample wells and control wells. The Developer Solution will turn blue in the presence of sufficient enzyme-substrate peptide binding.
11. Add 100 µl of Stop Solution to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding Stop Solution and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

## 8. Data Analysis

Calculate the Binding intensity of samples using the following formula:

Binding Intensity = Sample OD – Blank OD

### Histone Peptide Array Mapping:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Blank | K4m3 2 ng | K14m1 2 ng | K18m2 2 ng | K23m3 2 ng | K36m1 2 ng | K79m2 2 ng | K14ac 2 ng | K56ac 2 ng | H3R2m2s 2 ng | H3R17m1 2 ng | H3R26m2a 2 ng |
| B | Protein A/G | K4m3 20 ng | K14m1 20 ng | K18m2 20 ng | K23m3 20 ng | K36m1 20 ng | K79m2 20 ng | K14ac 20 ng | K56ac 20 ng | H3R2m2s 20 ng | H3R17m1 20 ng | H3R26m2a 20 ng |
| C | H3 protein | K9m1 2 ng | K14m2 2 ng | K18m3 2 ng | K27m1 2 ng | K36m2 2 ng | K79m3 2 ng | K18ac 2 ng | K79ac 2 ng | H3R8m1 2 ng | H3R17m2a 2 ng | H3R26m2s 2 ng |
| D | H3 protein | K9m1 20 ng | K14m2 20 ng | K18m3 20 ng | K27m1 20 ng | K36m2 20 ng | K79m3 20 ng | K18ac 20 ng | K79ac 20 ng | H3R8m1 20 ng | H3R17m2a 20 ng | H3R26m2s 20 ng |
| E | K4m1 2 ng | K9m2 2 ng | K14m3 2 ng | K23m1 2 ng | K27m2 2 ng | K36m3 2 ng | K4ac 2 ng | K27ac 2 ng | H3R2m1 2 ng | H3R8m2a 2 ng | H3R17m2s 2 ng | H3S10p 2 ng |
| F | K4m1 20 ng | K9m2 20 ng | K14m3 20 ng | K23m1 20 ng | K27m2 20 ng | K36m3 20 ng | K4ac 20 ng | K27ac 20 ng | H3R2m1 20 ng | H3R8m2a 20 ng | H3R17m2s 20 ng | H3S10p 20 ng |
| G | K4m2 2 ng | K9m3 2 ng | K18m1 2 ng | K23m2 2 ng | K27m3 2 ng | K79m1 2 ng | K9ac 2 ng | K36ac 2 ng | H3R2m2a 2 ng | H3R8m2s 2 ng | H3R26m1 2 ng | H3S28p 2 ng |
| H | K4m2 20 ng | K9m3 20 ng | K18m1 20 ng | K23m2 20 ng | K27m3 20 ng | K79m1 20 ng | K9ac 20 ng | K36ac 20 ng | H3R2m2a 20 ng | H3R8m2s 20 ng | H3R26m1 20 ng | H3S28p 20 ng |

## 9. Typical Data

Data provided for **demonstration purposes only.**

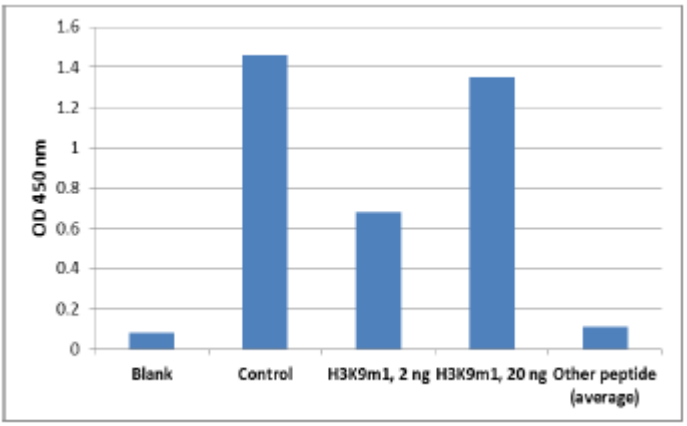



**Figure 1:** Graphical analysis of specificity and binding intensity of H3K9me1 antibody. The Histone H3 Array was probed with a H3K9me1 polyclonal antibody. Peptides and control were visualized using a goat anti-rabbit IgG-HRP secondary antibody.

# 10. Troubleshooting

| Problem | Possible cause | Suggestion |
|---|---|---|
| No signal or weak signal in both the positive control and sample wells | Reagents are added incorrectly. | Check if reagents are added in the proper order with the correct amount, and if any steps in the protocol may have been omitted by mistake. |
| | Incubation time and temperature are incorrect. | Ensure the incubation time and temperature described in the protocol are followed correctly. |
| | Incorrect absorbance reading. | Check if appropriate absorbance wavelength (450 nm) is used. |
| | Kit was not stored or handled properly. | Ensure all components of the kit were stored at the appropriate temperature and the cap is tightly capped after each opening or use. |
| High background present in the blank wells | Insufficient washing of wells. | Check if washing recommendations at each step are performed according to the protocol. |
| | Antibody concentration is too high. | Ensure the antibodies are appropriately diluted. |
| | Incubation time with Diluted Secondary is too long. | The incubation time with Diluted Secondary Antibody should not exceed 60 min. |
| | Over-development of color. | Decrease the development time with Developer Solution before adding Stop Solution. |
| No signal or weak signal only in sample wells | Antibody concentration is too low. | Ensure the antibodies are appropriately diluted. |
| | Sample amount added into the wells is insufficient. | Ensure a sufficient amount of sample antibodies or proteins or enzymes is added. |

| | | |
|---|---|---|
| | The primary antibody may have lost activity due to multiple freeze-thaw cycles, or bacterial contamination has changed the activity of an antibody. | Use new antibodies and avoid cross-contamination from other assays. |
| Uneven color development | Insufficient washing of the wells. | Ensure the wells are washed according to the guidance of washing and residual washing buffer is removed as much as possible. |
| | Delayed color development or delayed stopping of color development in the wells. | Ensure Developer Solution or Stop Solution is added sequentially and is consistent with the order you added the other reagents (e.g., from well A to well H or from well 1 to well 12). |

# 11. Notes

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