

**ab113452**

**EpiSeeker Histone**

**Methyltransferase**

**H3 (K4) Activity**

**Quantification Assay Kit**

Instructions for Use

For the measurement of activity/inhibition of H3-K4 specific histone methyltransferases using cell/tissue extracts

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



# Table of Contents

---

1. Overview	3
2. Background	3
3. Components and Storage	5
4. Protocol	7
5. Troubleshooting	11
6. Related Products	13

# 1. Overview

---

ab113452 is designed for measuring Histone Methyltransferases (HMT) that specifically target histone H3 at lysine 4. In this assay the histone substrate is stably captured on the strip wells. HMT enzymes transfer a methyl group to histone H3 substrate from Adomet to methylate the substrate at lysine 4. The methylated histone H3-K4 can then be recognized with a high-affinity antibody. The ratio or amount of methylated H3-K4, which is directly proportional to enzyme activity, can be quantified through HRP conjugated secondary antibody-color development system. The HMT activity is then calculated based on the amount of methylated H3-K4 converted by the HMTs.

# 2. Background

---

Histone methylation has been linked to a number of cellular processes including DNA repair, replication, transcriptional activation and repression. This process is regulated by Histone methyltransferases (HMTs), which are responsible for transferring methyl groups to the arginine and lysine residues of histone proteins. Lysine residues, which can be labelled with one, two or three methyl groups, are mainly modified in Histone H3 and histone H4.

Methylation of histone H3 at lysine 4 (H3-K4) is thought to be a global epigenetic mark in euchromatin and a mediator of activated

transcription. In mammalian cells, there are several HMTs that catalyze H3-K4 methylation, such as SET7/9 and MLL among other enzymes. Inhibition of HMTs could lead to the expression of silenced genes, which has led to the search of HMT inhibitors as potential therapeutic agents.

ab113452 provides a quick and efficient method to quantify HMT activity.

ab113452 has the following features:

- Procedure can be completed within 3 hours.
- Simple colorimetric assay
- Manual or high throughput: flexible assay format thanks to strip microplate
- Quantitative determination of activity/inhibition of H3-K4 histone methyltransferases.

### 3. Components and Storage

---

#### A. Kit Components

---

Item	Quantity (48 tests)	Quantity (96 tests)
EHM1 (10X Wash Buffer)	11 mL	22 mL
EHM2 (Histone Assay Buffer)	1.5 mL	3 mL
EHM3 (Adomet)*	25 µL	50 µL
EHM4 (Biotinylated Substrate, 25 µg/ml)*	100 µL	200 µL
EHM5 (HMT Standard, 10 µg/ml)*	10 µL	20 µL
EHM6 (Capture Antibody, 100 µg/ml)#	25 µL	50 µL
EHM7 (Detection Antibody, 200 µg/ml)*	10 µL	20 µL
EHM8 (Developing Solution)	6 mL	12 mL
EHM9 (Stop Solution)	3 mL	6 mL
Control Enzyme (200 µg/ml)*	5 µL	10 µL
8-Well Assay Strip (with frame)	6	12

---

\* For maximum recovery of the products, centrifuge the original vial after thawing prior to opening the cap.

# Sufficient volume of capture antibody is provided for 48 or 96 tests. Extra care need to be taken when preparing it. If the 48 or 96 tests are not performed at the same time, volume shortage of capture antibody can occur.

## **B. Additional Materials Required**

- Orbital shaker
- Pipettes and pipette tips
- Microplate reader
- 1.5 ml microcentrifuge tubes
- Distilled water

## **C. Storage**

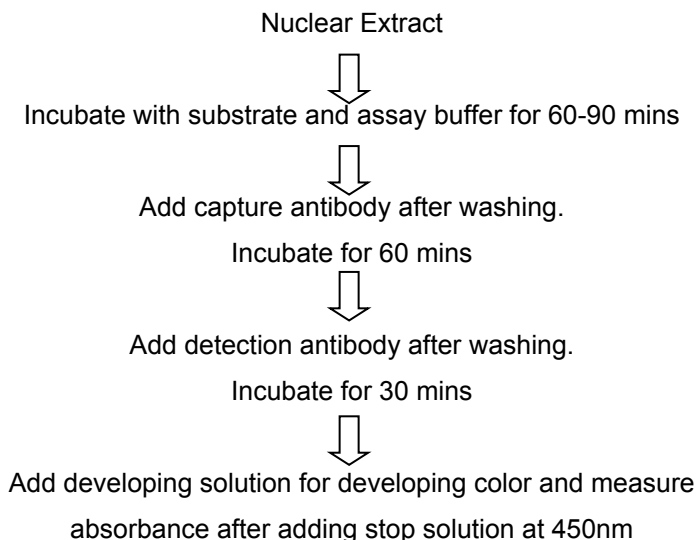
- Upon receipt store EHM3, EHM4, EHM5, EHM7, and the Control Enzyme at  $-20^{\circ}\text{C}$  away from light.
- Store all other components at  $4^{\circ}\text{C}$  away from light.
- The kit is stable for up to 6 months from the shipment date, when stored properly.

Note: Check if wash buffer, EHM1, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.

## 4. Protocol

---

### Protocol Summary



1. Prepare nuclear extracts by using your own successful method. For your convenience and the best results, Abcam offers a nuclear extraction kit (ab113474) optimized for use



with the EpiSeeker series. Nuclear extracts can be used immediately or stored at  $-80^{\circ}\text{C}$  for future use.

2. Determine the number of strip wells required. Leave these strips in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at  $4^{\circ}\text{C}$ ). Dilute EHM1 (10X Wash Buffer) with distilled water (pH 7.2-7.5) at a 1:10 ratio (ex: 1 ml of EHM1 + 9 ml of distilled water).
3. Dilute EHM3 with EHM2 (at a 1:5 ratio). Add 24  $\mu\text{l}$  of EHM2, 1.5  $\mu\text{l}$  of the diluted EHM3, and 2  $\mu\text{l}$  of EHM4 to each strip well. Then add 3  $\mu\text{l}$  of nuclear extracts (4-20  $\mu\text{g}$ ) or HMT enzymes, mix and cover the strip wells with Parafilm M and incubate at  $37^{\circ}\text{C}$  for 60-90 minutes.

For HMT inhibition, add 3  $\mu\text{l}$  of tested inhibitors at different concentrations and reduce EHM2 volume to 21  $\mu\text{l}$ . For the blank, add 3  $\mu\text{l}$  of EHM2 instead of nuclear extracts. For the standard curve, add 3  $\mu\text{l}$  of EHM2 instead of nuclear extracts, and add 2  $\mu\text{l}$  of EHM5 at different concentrations (ex: 0.1 – 5 ng/ $\mu\text{l}$ ) instead of EHM4. A positive control can be optionally set up by adding 0.5-1  $\mu\text{l}$  of the Control Enzyme and 2  $\mu\text{l}$  of EHM2 instead of nuclear extracts.

4. Aspirate and wash each well with 150  $\mu\text{l}$  of diluted EHM1 three times.

5. Dilute EHM6 (at a 1:100 ratio) to 1  $\mu\text{g/ml}$  with diluted EHM1. Add 50  $\mu\text{l}$  of diluted EHM6 to each strip well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).
6. Aspirate and wash each well with 150  $\mu\text{l}$  of diluted EHM1 five times.
7. Dilute EHM7 (at a 1:1000 ratio) with diluted EHM1. Add 50  $\mu\text{l}$  of the diluted EHM7 to each strip well and incubate at room temperature for 30 minutes.
8. Aspirate and wash each well with 150  $\mu\text{l}$  of diluted EHM1 five times.
9. Add 100  $\mu\text{l}$  of EHM8 into the wells and incubate at room temperature for 2-10 minutes away from light. Monitor the color development in the sample and standard wells (blue).
10. Add 50  $\mu\text{l}$  of EHM9 to each well to stop enzyme reaction when the color in the standard wells containing the higher concentrations of standard control turns medium blue. The color should change to yellow and absorbance can be read on a microplate reader at 450 nm within 2-15 minutes.
11. Calculate HMT activity or inhibition. For simple calculation use the following formula:

Activity (O.D./h/mg) =

$$\frac{\text{OD (no inhibitor – blank)}}{\text{Protein amount } (\mu\text{g})^* \times \text{hour}^{**}} \times 1000$$

Inhibition % =

$$1 - \left( \frac{\text{OD (inhibitor sample – blank)}}{\text{OD (no inhibitor control – blank)}} \right) \times 100\%$$

For accurate calculation, plot OD value versus amount of EHM5 and determine the slope as delta OD/ng and calculate HMT activity using the following:

Activity (ng/h/mg) =

$$\frac{\text{OD (sample – blank)}}{\text{Protein Amount } (\mu\text{g}) \times \text{hour} \times \text{slope}} \times 1000$$

\* Protein amount added into the reaction at step 3.

\*\* Incubation time at step 3.

## 5. Troubleshooting

---

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
No Signal for Both the Positive Control and the Samples	Reagents are added incorrectly.	Check if reagents are added in order and if any steps of the procedure may have been omitted by mistake.
	Incubation time and temperature is incorrect.	Ensure the incubation time and temperature in the protocol are followed correctly.
No Signal or Very Weak Signal for Only the Positive Control	The positive control enzyme is insufficiently added to the well.	Ensure a sufficient amount of control enzyme is added.
	The positive control enzyme has lost activity due to incorrect storage.	Follow the guidance in the protocol for storage of the positive control.
No Signal for Only the Sample	The protein sample is not properly extracted.	Ensure the nuclear protein extraction protocol is suitable for HMT protein extraction. Sodium chloride concentration of the extraction buffer should not be more than 100 mM
	The protein amount is added into well insufficiently.	Ensure extract contains a sufficient amount of protein.

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
No Signal for Only the Sample(cont'd)	The sample is not prepared from fresh cells or tissues.	The nuclear extracts from frozen cells or tissues significantly lose enzyme activity. Fresh samples should be used.
	Nuclear extracts are incorrectly stored or have been stored for a long duration.	Ensure the nuclear extracts are stored at $-80^{\circ}\text{C}$ for no more than 6 weeks.
High Background Present for the Blank	The well is not washed sufficiently.	Check if wash at each step is performed according to the protocol.
	Contaminated by the positive control of HMT standard.	Ensure the well is not contaminated from adding the control enzyme or HMT standard accidentally, or from using enzyme or HMT standard contaminated tips.
	Overdevelopment.	Decrease development time in protocol step 9.

**For further technical questions please do not hesitate to contact us by email ([technical@abcam.com](mailto:technical@abcam.com)) or phone (select “*contact us*” on [www.abcam.com](http://www.abcam.com) for the phone number for your region).**

## 6. Related Products

---

- EpiSeeker Nuclear Extraction Kit (ab113474)
- EpiSeeker Histone Methyltransferase H3 (K9) Activity Quantification Assay Kit (ab113453)
- EpiSeeker Histone Methyltransferase H3 (K27) Activity Quantification Assay Kit (ab113454)



**For all technical and commercial enquires  
please go to:**

[www.abcam.com/contactus](http://www.abcam.com/contactus)

[www.abcam.cn/contactus](http://www.abcam.cn/contactus) (China)

[www.abcam.co.jp/contactus](http://www.abcam.co.jp/contactus) (Japan)