

ab113453 – Histone Methyltransferase H3 (K9) Activity Quantification Assay Kit

Instructions for Use

For the measurement of activity/inhibition of H3K9 specific histone methyltransferases in cell/tissue extracts

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

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1. 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 9 (H3K9) is thought to define silent, transcriptionally inactive chromatin as it seems to inhibit other processes such as H3/H4 acetylation. In mammalian cells, there are several HMTs that catalyze H3K9 methylation, such as SuVar39h and ESET 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.

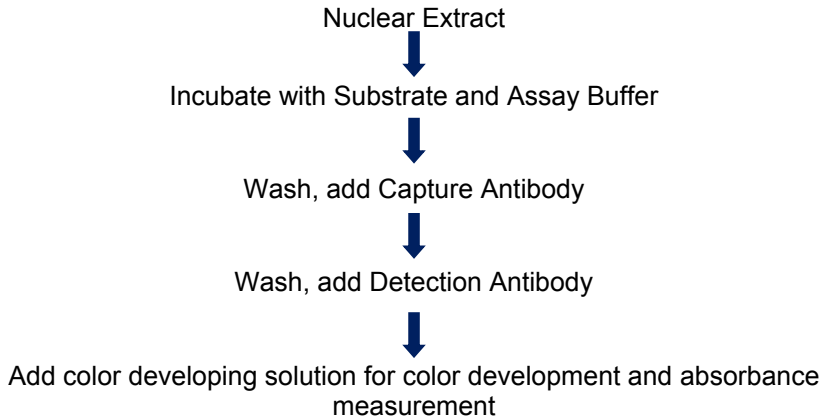
ab113453 provides a quick and efficient method to quantify HMT activity.

The kit 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-K9 histone methyltransferases

The Histone Methyltransferase H3 (K9) Activity Quantification Assay Kit is designed for measuring Histone Methyltransferases (HMTs) that specifically target histone H3 at lysine 9. In an assay with this kit, 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 9. The methylated histone H3K9 can be recognized with a high-affinity antibody. The ratio or amount of methylated H3K9, 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 H3K9 converted by the HMTs.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit as given in the table upon receipt and away from light.

Observe the storage conditions for individual prepared components in sections 9 & 10. The kit is stable for up to 6 months from the shipment date, when stored properly.

For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

Check if Wash and Antibody Buffers contain salt precipitates before use. If so, warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.

5. MATERIALS SUPPLIED

Item	Quantity (48 tests)	Quantity (96 tests)	Storage Condition (Before Preparation)
10X Wash Buffer	11 mL	22 mL	4°C
Histone Assay Buffer	1.5 mL	3 mL	4°C
Adomet*	25 µL	50 µL	-20°C
Biotinylated Substrate, 25 µg/mL*	100 µL	200 µL	-20°C
HMT Standard, 10 µg/mL*	10 µL	20 µL	-20°C
Capture Antibody, 100 µg/ mL*	25 µL	50 µL	4°C
Detection Antibody, 200 µg/mL*	10 µL	20 µL	-20°C
Developing Solution	6 mL	12 mL	4°C
Stop Solution	3 mL	6 mL	4°C
Control Enzyme (300 µg/ mL)*	5 µL	10 µL	-20°C
8-Well Assay Strip (with frame)	6	6	4°C

*Spin the solution down to the bottom prior to use.

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

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Orbital shaker
- Pipettes and pipette tips
- Reagent reservoir
- 1.5 mL microcentrifuge tubes
- Microplate reader

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Prepare fresh reagents immediately prior to use.

9.1 **1X Wash Buffer**

Dilute 10X Wash Buffer with distilled water (pH 7.2-7.5) at a 1:10 ratio (1 mL of 10X Wash Buffer + 9 mL of water) to make 1X Wash Buffer.

9.2 **Adomet Solution**

Dilute Adomet with Histone Assay Buffer (at a 1:5 ratio).

9.3 **Capture Antibody**

Dilute Capture Antibody (at a 1:100 ratio) to 1 µg/mL with 1X Wash Buffer.

9.4 **Detection Antibody**

Dilute Detection Antibody (at a 1:1000 ratio) with 1X Wash Buffer.

9.5 **HMT Standard**

Dilute HMT Standard over a range of 0.1-5 ng/µL.

10. SAMPLE PREPARATION

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 this Kit. Nuclear extracts can be used immediately or stored at -80°C for future use.

11. ASSAY PROCEDURE

- 11.1 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°C).
- 11.2 Add 24 μL of Histone Assay Buffer, 1.5 μL of the diluted Adomet, and 2 μL of Biotinylated Substrate to each strip well. Then add 3 μL of nuclear extracts (4-20 μg) or HMT enzymes, mix and cover the strip wells with Parafilm M and incubate at 37°C for 60-90 minutes. For HMT inhibition, add 3 μL of tested inhibitors at different concentrations and reduce Histone Assay Buffer volume to 21 μL . For the blank, add 3 μL of Histone Assay Buffer instead of nuclear extracts. For the standard curve, add 3 μL of Histone Assay Buffer instead of nuclear extracts, and add 2 μL of HMT Standard at different concentrations (e.g. 0.1 - 5 $\text{ng}/\mu\text{L}$, see 9.5) instead of Biotinylated Substrate. A positive control can be optionally set up by adding 0.5-1 μL of the Control Enzyme and 2 μL of Histone Assay Buffer instead of nuclear extracts.
- 11.3 Aspirate and wash each well with 150 μL of 1X Wash Buffer three times.
- 11.4 Add 50 μL of diluted Capture Antibody to each strip well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).
- 11.5 Aspirate and wash each well with 150 μL of diluted 1X Wash Buffer five times.
- 11.6 Add 50 μL of the diluted Detection Antibody to each strip well and incubate at room temperature for 30 minutes.
- 11.7 Aspirate and wash each well with 150 μL of diluted 1X Wash Buffer five times.
- 11.8 Add 100 μL of Developing Solution 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).
- 11.9 Add 50 μL of Stop Solution 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

12. ANALYSIS

Calculate HMT activity or inhibition.

For simple calculation use the following formula:

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

$$\frac{\text{No inhibitor OD} - \text{Blank OD}}{\text{Protein amount } (\mu\text{g})^* \times \text{hour}^{**}} \times 1000$$

* Protein amount added into the reaction at step 11.2.

** Incubation time at step 11.2.

Inhibition % =

$$1 - \left(\frac{\text{Inhibitor sample OD} - \text{Blank OD}}{\text{No inhibitor control OD} - \text{Blank OD}} \right) \times 100\%$$

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

Activity (ng/h/mg) =

$$\frac{\text{Sample OD} - \text{Blank OD}}{\text{Protein Amount } (\mu\text{g}) \times \text{hour} \times \text{slope}} \times 1000$$

13. TROUBLESHOOTING

Problem	Cause	Solution
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 are 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

	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°C for no more than 6 weeks
	Absence of HMT activity in the sample due to treatment	N/A
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 11.8

14. Notes



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