ab113455 – Histone Demethylase (H3K4) Activity Quantification Assay Kit

Instructions for Use

For the measurement of activity/inhibition of histone demethylases (H3K4 specific) in cell/tissue extracts

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

Version 1 Last Updated 1 September 2014

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1. BACKGROUND

Lysine histone methylation is one of the most robust epigenetic marks and is essential for the regulation of multiple cellular processes. The methylation of H3K4 seems to be of particular significance, as it is associated with active regions of the genome. H3K4 methylation was considered irreversible until the identification of a large number of histone demethylases indicated that demethylation events play an important role in histone modification dynamics. So far, at least 2 classes of H3K4 specific histone demethylase, LSD1 and JARIDs, have been identified. LSD1 can remove di- and mono-methylation from H3K4 by using an amine oxidase reaction, while JARIDs such as RBP2, PLU-1, SMCX, and SMCY catalyzes the removal of methylation by using a hydroxylation reaction and required iron and α-ketoglutarate as cofactors. H3K4 specific demethylases are found to be involved in some pathological processes such as cancer progression. Inhibition of the enzymes may lead to re-methylation of H3K4 and silencing of H3K4 enriched active genes. There are few methods currently available for measuring activity/inhibition of H3K4 specific methylases using a variety of cells/tissues.

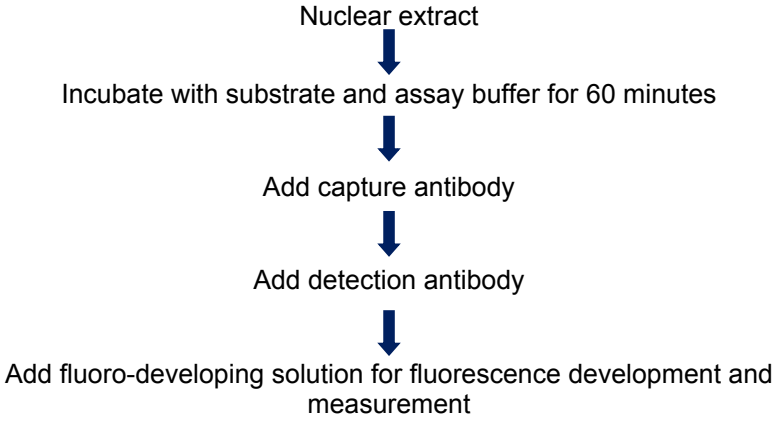
ab113455 uses a proprietary and unique procedure to measure activity/inhibition of H3K4 specific histone demethylases using cell/tissue extracts.

This kit has the following features:

- Fast procedure, which can be finished within 3 hours
- Innovative fluorescent assay without the need for radioactivity, extraction, or chromatography
- Direct measurement of HDM (H3K4 specific) activity and inhibition without interference by thiol-containing chemicals such as DTT, GSH, and 2-mercaptoethanol
- Strip microplate format makes the assay flexible: manual or high throughput analysis
- Simple, reliable, and consistent assay conditions

ab113455 is designed for measuring total histone demethylase (H3K4 specific) activity/inhibition. In the assay with this kit, the unique di-methylated histone H3K4 substrate is stably captured on the strip wells. Active Histone demethylases (HMDs) bind to and demethylate histone H3K4 substrate. The remaining un-demethylated substrate can be recognized with a high affinity anti-methylated histone H3K4 antibody. The ratio or amount of the un-demethylated histone, which is inversely proportional to HDM enzyme activity, can then be fluorometrically quantified.

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 and away from light upon receipt.

Observe the storage conditions for individual prepared components in sections 9 & 10.

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

Check if the 10X Wash Buffer contains 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	48 Tests	96 Tests	Storage Condition (Before Preparation)
10X Wash Buffer	15 mL	30 mL	4°C
HDM Assay Buffer	1.5 mL	3 mL	4°C
HDM Substrate	50 µL	100 µL	-20°C
HDM Standard, 20 µg/mL	25 µL	50 µL	-20°C
Capture Antibody, 1000 µg/mL	5 µL	10 µL	4°C
Detection Antibody, 200 µg/mL	8 µL	16 µL	-20°C
Fluoro Developer	12 µL	24 µL	-20°C
Fluoro Enhancer	12 µL	24 µL	4°C
Fluoro Dilution	4 mL	8 mL	4°C
8-Well Assay Strip (with Frame)	6	12	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

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

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

All reagents provided are ready to use.

10. SAMPLE PREPARATION

Prepare nuclear extracts by using you own successful method. For your convenience and the best results, Abcam offers a nuclear extraction kit (ab113474) for your convenience. Nuclear extracts can be used immediately or stored at -80°C for future use.

11. ASSAY PROCEDURE

11.1 Predetermine the number of strip wells required. Remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C). Dilute the 10X Wash Buffer with distilled water (pH 7.2-7.5) at a 1:10 ratio to make 1X Wash Buffer (e.g. 1 mL of 10X Wash Buffer + 9 mL of water).

11.2 Dilute HDM Substrate at a 1:50 ratio with 1X Wash Buffer, and add 50 µL of the Diluted HDM Substrate into each well apart from blank and standard wells. For preparation of the standard curve, add 50 µL of 1X Wash Buffer into the wells (no HDM Substrate added), followed by adding 1 µL of HDM Standard at different concentrations (0.1-10 ng). Cover the wells with Parafilm M and incubate at room temperature for 30-45 minutes.

11.3 Aspirate and wash the wells with 150 µL of 1X Wash Buffer two times.

11.4 For Sample wells: Add 28 µL of HDM Assay Buffer and 2 µL of nuclear extracts (4-20 µg) to each sample well.
For Control wells: Add 30 µL of HDM Assay Buffer to each well.
For HDM Inhibition wells: Add tested inhibitors at different amounts and reduce HDM Assay Buffer volume for a total volume of 30 µL.
For Blank wells: Add 30 µL of HDM Assay Buffer into the blank wells (no HDM Substrate added).
Mix, cover the strip wells, and incubate at 37°C for 60 minutes.

Note: *The standard wells can be left dry for this step and should not contain HDM Assay Buffer or nuclear extract.*

11.5 Aspirate and wash the wells with 150 µL of 1X Wash Buffer three times.

11.6 Dilute the Capture Antibody (at a 1:1000 ratio) to 1 µg/mL with 1X Wash Buffer. Add 50 µL of the Diluted Capture Antibody to each strip well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).

11.7 Aspirate and wash each well with 150 µL of 1X Wash Buffer four times.

11.8 Dilute the Detection Antibody (at a 1:1000 ratio) to 0.2 µg/mL with 1X Wash Buffer. Add 50 µL of the Diluted Detection Antibody to each strip well and incubate at room temperature for 25-30 minutes.

11.9 Aspirate and wash each well with 150 µL of 1X Wash Buffer five to six times.

11.10 Prepare fluoro-development solution by adding 1 µL of Fluoro Developer and 1 µL of Fluoro Enhancer into each 400 µL of Fluror Dilution. Add 50 µL of fluoro-development solution into the wells and incubate at room temperature for 2-5 minutes away from light. Measure and read fluorescence on a fluorescence microplate reader at Ex/Em = 530/590 nm.

Note: *If the strip well frame does not fit the fluorescence reader, transfer the solution to a standard 96-well microplate and read fluorescence at Ex/Em = 530/590 nm.*

11.11 Calculate HDM (H3K4) activity or inhibition using the formulae provided in Section 13 – Data Analysis.

12. ANALYSIS

Calculate the HDM (H3K4) activity or inhibition using the following simple calculation:

HDM activity (RFU/h/µg) =

$$\frac{\text{(Control RFU – Blank RFU) – (Sample RFU – Blank RFU)}}{\text{Reaction time (0.5 – 1 hour) x protein amount added (µg)}}$$

Inhibition % =

$$1 - \left(\frac{\text{(Control RFU – Blank RFU) – (Inhibitor Sample RFU – Blank RFU)}}{\text{(Control RFU – Blank RFU) – (No Inhibitor Sample RFU – Blank RFU)}} \right) \times 100\%$$

For an accurate calculation, plot delta RFU value versus amount of HDM Standard and determine the slope as delta RFU/ng.

Calculate HDM (H3K4) activity using the following formula:

Amount (ng/h/µg) =

$$\frac{\text{(Control RFU – Blank RFU) – (Sample RFU – Blank RFU)}}{\text{Slope x Reaction time (1 hour) x Protein amount added (µg)}} \times 1000$$

13. TROUBLESHOOTING

Problem	Cause	Solution
No Signal for the Sample	The protein sample is not properly extracted	Ensure the protein extraction protocol is suitable for nuclear protein extraction
	The protein amount is added into well insufficiently	Ensure extract contains a sufficient amount of protein
	The sample is prepared from frozen cells or tissues	The nuclear extracts from frozen cells/tissue significantly lose enzyme activity. A fresh sample should be used
	Nuclear extracts are incorrectly stored	Ensure the nuclear extracts are stored at -80°C
	Reagents are added incorrectly	Check if reagents are added in order and if any steps of the procedure have been omitted by mistake
	Incubation time and temperature is incorrect	Ensure the incubation time and temperature described in the protocol is followed correctly
	Absence of HDM (H3K4) 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
	Overdevelopment	Decrease development time

14. NOTES

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