ab115086 – Histone H3 (di-methyl K79) Quantification Kit (Fluorometric)

Instructions for Use

For the measurement of global H3K79me2

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

Version 1a Last Updated 22 February 2019

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1. BACKGROUND

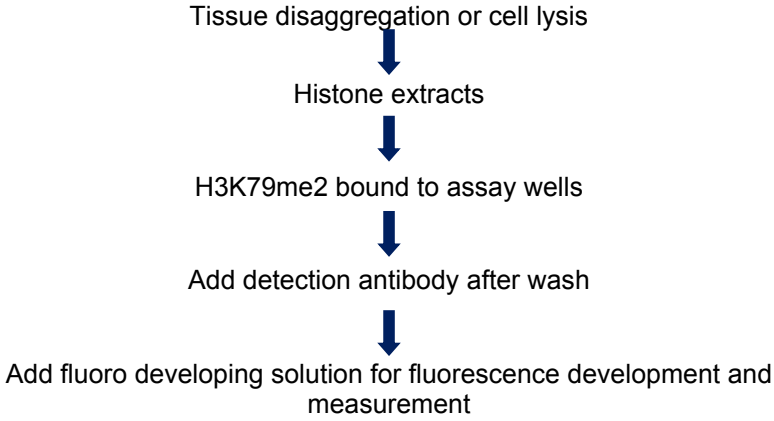
Epigenetic activation or inactivation of genes plays a critical role in many important human diseases, especially in cancer. A major mechanism for epigenetic inactivation of the genes is methylation of CpG islands in genome DNA caused by DNA methyltransferases. Histone methyltransferases (HMTs) control or regulate DNA methylation through chromatin-dependent transcription repression or activation. HMTs transfer 1-3 methyl groups from S-adenosyl-L-methionine to the lysine and arginine residues of histone proteins. Dot1 is a histone methyltransferase that catalyzes methylation of histone H3 at lysine 79 (H3K79) in mammalian cells. H3K79 di-methylation is a widespread histone modification and is associated with transcriptionally active genes. Increased global H3K79 di-methylation is also found to be involved in some pathological processes such as leukemogenesis in human. The global H3K79 di-methylation can also be changed by inhibition or activation of HMTs. Thus, quantitative detection of global H3K79me2 would provide useful information for better understanding epigenetic regulation of gene activation and for developing HMT-targeted drugs.

ab115086 provides a tool for measuring global di-methylation of histone H3K79. The kit has the following features:

- Quick and efficient procedure, which can be finished within 2.5 hours
- Innovative fluorometric assay without the need for radioactivity, electrophoresis, or chromatography
- Specifically captures H3K79me2 with the detection limit as low as 0.4 ng/well and detection range from 5 ng-2 µg/well of histone extracts
- The control is conveniently included for the quantification of the amount of H3K79me2
- Strip microplate format makes the assay flexible: manual or high throughput
- Simple, reliable, and consistent assay conditions

The Histone H3 (di-methyl K79) Quantification Kit (Fluorometric) is designed for measuring global histone H3K79 di-methylation. In an assay with this kit, the di-methylated histone H3 at lysine 79 is captured to the strip wells coated with an anti-H3K79me2 antibody. The captured H3K79me2 can then be detected with a labeled detection antibody, followed by a fluorescent development reagent. The ratio H3K79me2 is proportional to the intensity of fluorescence. The absolute amount of H3K79me2 can be quantified by comparing to the standard control.

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.

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

Check if the 10X Wash Buffer and Antibody Buffer 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	10 mL	20 mL	4°C
Antibody Buffer	6 mL	12 mL	4°C
Detection Antibody, 1 mg/mL	5 µL	10 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
Standard Control, 100 µg/mL	10 µL	20 µL	-20°C
8-Well Assay Strip (with Frame)	4	9	4°C
8-Well Standard Control Strips*	2	3	4°C

* These have a green rim around the wells to help with identification.

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
- Fluorescence microplate reader
- 15 mL conical tube
- 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

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 (e.g. 1 mL of 10X Wash Buffer + 9 mL of water).

9.2 **Diluted Detection Antibody**

Dilute Detection Antibody (at a 1:1000 ratio) to 1 µg/mL with Antibody Buffer.

10. SAMPLE PREPARATION

Prepare histone extracts from cells/tissues treated or untreated by using your own successful method (acid extraction or high salt extraction). For your convenience and the best results, Abcam offers the Histone Extraction Kit (ab113476) optimized for use in Abcam's modified histone quantification series. Alternatively, preparation of histone extracts can also be performed using the procedure below:

10.1 For tissues (treated and untreated), weigh the sample and cut the sample into small pieces (1-2 mm^3) with a scalpel or scissors. Transfer tissue pieces to a Dounce homogenizer. Add TEB buffer (PBS containing 0.5% Triton X 100, 2 mM PMSF and 0.02% NaN_3) at 200 mg/mL, and disaggregate tissue pieces by 50-60 strokes. Transfer homogenized mixture to a 15 mL conical tube and centrifuge at 3000 rpm for 5 minutes at 4°C. If total mixture volume is less than 2 mL, transfer mixture to a 2 mL vial and centrifuge at 10,000 rpm for 1 minute at 4°C. Remove supernatant.

For cells (treated and untreated), harvest cells and pellet the cells by centrifugation at 1000 rpm for 5 minutes at 4°C. Resuspend cells in TEB buffer at 10^7 cells/mL and lyse cells on ice for 10 minutes with gentle stirring. Centrifuge at 3000 rpm for 5 minutes at 4°C. If total volume is less than 2 mL, transfer cell lysates to a 2 mL vial and centrifuge at 10,000 rpm for 1 minute at 4°C. Remove supernatant.

10.2 Resuspend cell/tissue pellet in 3 volumes (approx. 200 µL/10^7 cells or 200 mg tissues) of extraction buffer (0.5N HCl + 10% glycerol) and incubate on ice for 30 minutes.

10.3 Centrifuge at 12,000 rpm for 5 minutes at 4°C and remove the supernatant fraction to a new vial.

10.4 Add 8 volumes (approx. 0.6 mL/10^7 cells or 200 mg tissues) of acetone and leave at -20°C overnight.

10.5 Centrifuge at 12,000 rpm for 5 minutes and air-dry the pellet. Dissolve the pellet in distilled water (30-50 µL/10^7 cells or 200 mg tissues).

10.6 Quantify the protein concentration. Aliquot the extract and store the extract at -20°C or -80°C.

Histone 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 50 µL of Antibody Buffer into each well. For the sample, add 1-2 µg of the histone extract into the sample wells. For the standard curve, dilute Standard Control with Antibody Buffer to 1-100 ng/µL at 5-7 points (e.g. 1.5, 3, 6, 12, 25, 50, and 100 ng/µL). Add 1 µL of Standard Control at the different concentrations into the Standard Control Wells (marked with green rims). For the blank, do not add any nuclear extracts or standard control protein. Mix and cover the strip wells with Parafilm M and incubate at room temperature for 1-2 hours.

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

11.4 Add 50 µL of diluted Detection Antibody to each well and incubate at room temperature for 60 minutes on an orbital shaker (100 rpm).

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

11.6 Prepare fluoro-development solution by adding 1 µL of Fluoro Developer and 1 µL of Fluoro Enhancer into each 400 µL of Fluoro Diluter. Add 50 µL of fluoro-development solution into the wells and incubate at room temperature for 1-5 minutes away from light. The color in the standard wells containing the higher concentrations may turn slightly pink during this period. 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.*

12. ANALYSIS

Calculate % H3K79me2:

$$\text{H3K79me2 \%} = \frac{\text{Treated (Tested) Sample RFU} - \text{Blank RFU}}{\text{Untreated (Control) Sample RFU} - \text{Blank RFU}} \times 100\%$$

For the amount quantification plot OD versus amount of Standard Control and determine the slope as delta OD/ng.

Calculate the amount of H3K27me using the following formula:

$$\text{Amount (ng/mg protein)} = \frac{\text{Sample RFU} - \text{Blank RFU}}{\text{Protein (µg)*} \times \text{Slope}} \times 1000$$

*Histone extract amount added into the sample well at step 11.2.

13. TROUBLESHOOTING

Problem	Cause	Solution
No Signal for Both the Standard Control and the Samples	Reagents are added incorrectly	Check if reagents are added in order and if some steps of the procedure are omitted by mistake
	Incubation time and temperature is incorrect	Ensure the incubation time and temperature described in the protocol is followed correctly
No Signal or Very Weak Signal for Only the Standard Control	The amount of Standard Control is not added into the standard control wells or is added insufficiently	Ensure a sufficient amount of control is added to the well
No Signal for Only the Sample	The protein sample is not properly extracted	Ensure the procedure and reagents are correct for the nuclear protein extraction
	The protein amount is added into well insufficiently	Ensure extract contains a sufficient amount of protein
	Protein extracts are incorrectly stored	Ensure the protein extracts are stored at -20°C or -80°C
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 Standard control	Ensure the well is not contaminated from adding the control protein or by using control protein contaminated tips
	Overdevelopment	Decrease development time in step 11.6

14. NOTES

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