

ab115071 – Histone H3 (di-methyl K27) Quantification Kit (Fluorometric)

Instructions for Use

For the measurement of global histone H3K27 di-methylation using a variety of mammalian cells including fresh and frozen tissues, and cultured adherent and suspension cells

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

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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. G9a and polycomb group enzymes such as EZH2 are histone methyltransferases that catalyze methylation of histone H3 at lysine 27 (H3K27) in mammalian cells. Histone H3K27 di-methylation, a modification enriched at pericentromeric heterochromatin, is observed to be broadly distributed throughout all euchromatic sites and participates in silencing gene expression. Increased global H3K27 di- and tri-methylation is found to be involved in some pathological processes such as cancer progression. The global H3K27 di-methylation can be also changed by inhibition or activation of HMTs. Thus quantitative detection of global di-methyl histone H3K27 would provide useful information for better understanding epigenetic regulation of gene activation/repression and for developing HMT-targeted drugs.

ab115071 provides a tool for measuring global di-methylation of histone H3K27.

This kit has the following features:

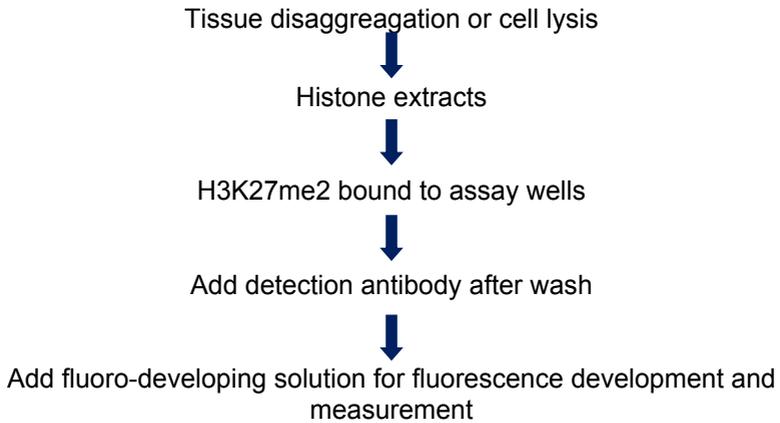
- Quick and efficient procedure, which can be finished within 2.5 hours
- Innovative fluorometric assay without the need for radioactivity, electrophoresis, or chromatography
- Specifically captures H3K27me₂ with the detection limit as low as 0.4 ng/well and detection range from 5 ng-2 µg/well of histone extracts

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- The control is conveniently included for the quantification of the amount of H3K27me2
- Strip microplate format makes the assay flexible: manual or high throughput
- Simple, reliable, and consistent assay conditions

Abcam's Histone H3 (di-methyl K27) Quantification Kit (Fluorometric) is designed for measuring global histone H3K27 di-methylation. In an assay with this kit, the di-methylated histone H3 at lysine 27 is captured to the strip wells coated with an anti-H3K27me2 antibody. The captured di-methylated histone H3K27 can then be detected with a labeled detection antibody, followed by a fluorescent development reagent. The ratio of H3K27me2 is proportional to the intensity of fluorescence. The absolute amount of H3K27me2 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 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 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 | 48 Tests | 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 wells are identified by a green ring around the top.

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
- Reagent reservoirs
- Orbital shaker
- 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

All reagents provided are ready to use.

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 mm³) 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% NaN₃) 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 10⁷ 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/10⁷ 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/10⁷ 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/10⁷ 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. PLATE PREPARATION

- Strip 1-3 (for 96 assays) or strip 1-2 (for 48 assays) – standard wells (green trimmed); the standard curve can be generated with 5-8 concentration points (includes blank).
- Example amount of standard control/well - A1: 100 ng; B1: 50 ng; C1: 25 ng; D1: 12 ng; E1: 6 ng; F1: 3 ng; G1: 1.5 ng; H1: 0 ng.
- Strip 4-12 (for 96 assays) or strip 3-6 (for 48 assays) – sample wells (No label).
- Each sample or standard point can be assayed in duplicates or triplicates.

12. ASSAY PROCEDURE

- 12.1 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).
 - 12.2 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 the 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 wells (ringed in green). 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.
 - 12.3 Aspirate and wash the wells with 150 μL of 1X Wash Buffer three times.
 - 12.4 Dilute the Detection Antibody (at 1:1000 ratio) to 1 $\mu\text{g}/\text{mL}$ with Antibody Buffer. Add 50 μL of Diluted Detection Antibody to each well and incubate at room temperature for 60 minutes on an orbital shaker (100 rpm).
 - 12.5 Aspirate and wash the wells with 150 μL of 1X Wash Buffer six times.
 - 12.6 Prepare fluoro-development solution by adding 1 μL of Fluoro Developer and 1 μL of Fluoro Enhancer into each 400 μL of Fluoro Dilution. 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.*
- 12.7 Calculate % histone H3K27 di-methylation using the formulae provided in Section 13 – Data Analysis.

13. ANALYSIS

Calculate the % Histone H3K27 di-methylation using the following formula:

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

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

Calculate the amount of H3K27me2 using the following formula:

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

*Histone extract amount added into the sample well at step 12.2.

14. 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 12.6 |

15. NOTES

RESOURCES

UK, EU and ROW

Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria

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France

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Switzerland

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