ab115045 – Histone H3 (tri-methyl K27) Assay Kit (In Situ)

Instructions for Use

For the measurement of in situ tri-methylation of histone H3K27

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

Version 1 Last Updated 3 September 2014

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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 enzyme such as EZH2 are histone methyltransferases that catalyze methylation of histone H3 at lysine 27 (H3K27) in mammalian cells. Histone H3K27me3 is a facultative heterochromatin mark, which promotes the recruitment of polycomb group proteins for gene silencing. Increased global histone H3K27me3 is also found to be involved in some pathological processes such as cancer progression. There are only a couple of methods such as western blot, which are used for measuring histone H3K27me3. The methods available so far are time consuming and labor intensive, or have low throughput.

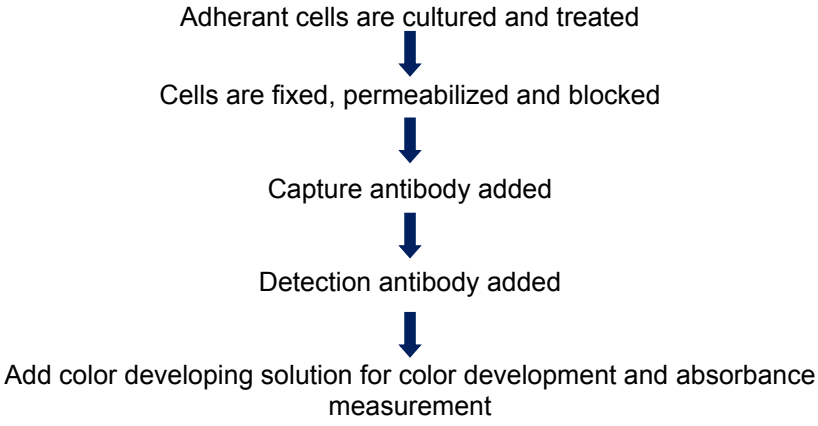
The Histone H3 (tri-methyl K27) Assay Kit (In Situ) addresses these problems by using a unique procedure to measure *in situ* histone H3K27me3.

The kit has the following features:

- Quick and efficient procedure, which can be finished within 3 hours
- Innovative colorimetric assay without the need for radioactivity, electrophoresis, or chromatography
- Measurement of *in situ* H3K27me3 without the need to prepare cell lysates
- Microplate format makes the assay suitable for high throughput analysis of agents that increases or inhibits H3K27me3
- Simple, reliable, and consistent assay conditions

Abcam's Histone H3 (tri-methyl K27) Assay Kit (In Situ) is a whole cell-based detection of H3K27me3. In this assay, adherent cells are cultured in conventional 96-well microplates. After your experimental treatment, cells are fixed and permeabilized. The H3K27me3 is then detected by an anti-H3K27me3 antibody. The ratio or amount of H3K27me3 can be quantified through HRP conjugated secondary antibody-color development system and is proportional to the intensity of color development.

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 Permeabilizing 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 (96 tests)	Quantity (2 x 96 tests)	Storage Condition (Before Preparation)
10X Wash Buffer	30 mL	2 x 30 mL	4°C
Permeabilizing Buffer	30 mL	2 x 30 mL	4°C
Blocking Buffer	20 mL	2 x 20 mL	4°C
Antibody Buffer	10 mL	20 mL	4°C
Capture Antibody, 1000 µg/mL	9 µL	18 µL	4°C
Detection Antibody, 200 µg/mL	10 µL	20 µL	-20°C
Developing Solution	12 mL	24 mL	4°C
Stop Solution	6 mL	12 mL	4°C
30% H_2O_2 Solution	0.5 mL	1 mL	4°C
H3K27me3 Control, 20 µg/mL	15 µL	30 µL	-20°C
8-Well Control Strips	2	4	4°C
Microplate	1	2	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Culture media
- Pipettes and pipette tips
- Microplate reader
- 15 mL Conical Tube
- 1.5 mL Microfuge Tubes
- 37% Formaldehyde
- Orbital Shaker
- Adsorbant paper
- PBS

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 **Fixing Solution**

Add 2.16 mL of 37% Formaldehyde to 18 mL of PBS.

9.3 **1% Hydrogen Peroxide**

Add 330 µL of 30% H_2O_2 Solution into 10 mL of Permeabilizing Buffer.

9.4 **Capture Antibody Solution**

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

9.5 **Detection Antibody Solution**

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

10. SAMPLE PREPARATION

Inoculate and grow adherent cells in a 96-well microplate to 50-70% confluency. Leave 2-4 wells with no cell inoculation as the blank.

Treat cells with appropriate amount of agents that may increase or reduce H3K27me3 for appropriate time.

11. ASSAY PROCEDURE

11.1 Remove culture media from the wells with a wrist-flick.

11.2 Immediately add 150 µL of Fixing Solution slowly to the wells and incubate at room temperature for 15 minutes. Remove fixing solution from wells with a wrist-flick; while still inverted, tap the plate gently onto the absorbent paper to remove any excess fixing agent still within the wells.

11.3 Wash wells once (for 2 minutes) with 150 µL of 1X Wash Buffer.

11.4 Remove wash buffer with a wrist flick; while still inverted, tap the plate onto the absorbent paper to remove any excess solution. Add 150 µL of Permeabilizing Buffer to each well and incubate at room temperature for 5 minutes.

11.5 Remove Permeabilizing Buffer from the wells with a wrist flick. Add 100 µL of the 1% Hydrogen Peroxide Solution into each well and incubate at room temperature for 10 minutes to remove endogenous peroxidase.

11.6 Remove the H_2O_2 solution from wells with a wrist flick and wash the wells twice with 150 µL of 1X Wash Buffer.

11.7 Remove wash buffer with a wrist flick; while still inverted, tap the plate onto the absorbent paper to remove any excess solution. Add 150 µL of Blocking Buffer to the wells and incubate at 37°C for 45 minutes. Meanwhile, add 50 µL of 1X Wash Buffer to the desired number of Control Strip wells, followed by adding 1 µL of H3K27me3 Control at the different amounts (e.g. 0.5-20 ng, diluted with distilled water) and incubate at room temperature for 30-45 minutes. For the blank wells, do not add any H3K4me3 Control.

11.8 Remove Blocking Buffer with a wrist flick; while still inverted, tap the plate onto the absorbent paper. Wash the wells twice with 150 µL of 1X Wash Buffer. For each wash, remove the wash buffer with a wrist flick; while still inverted, tap the plate onto the absorbent paper to remove any excess solution. Meanwhile, aspirate the solution from the control strip wells and wash the wells with 150 µL of 1X Wash Buffer three times.

11.9 Add 50 µL of diluted Capture Antibody to the sample wells and H3K27me3 control strip wells. Incubate at room temperature for 60 minutes on an orbital shaker (100 rpm).

11.10 Remove solution from the wells with a wrist flick and wash the wells four times with 150 µL of 1X Wash Buffer. For each wash, remove the wash buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper.

11.11 Add 50 µL of diluted Detection Antibody to the wells and incubate at room temperature for 30 minutes.

11.12 Remove solution from the wells with a wrist flick and wash the wells four times with 150 µL of 1X Wash Buffer. For each wash, remove the wash buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper.

11.13 Add 100 µL of Developing Solution to the wells and incubate at room temperature for 2-10 minutes away from light. Monitor the color development in the sample and control wells (blue).

11.14 Add 50 µL of Stop Solution to the wells and read absorbance on a microplate reader at 450 nm.

12. ANALYSIS

Calculate % H3K27me3:

$$\text{H3K27me3 \%} = \frac{\text{Treated (Tested) Sample OD – Blank OD}}{\text{Untreated (control) sample OD – blank OD}} \times 100\%$$

For accurate calculation, plot OD value versus amount of H3K27me3 Control and determine the slope as delta OD/ng.

Calculate the amount of H3K27me3 using the following formula:

$$\text{H3K27me3 (ng)} = \frac{\text{Sample OD – Blank OD}}{\text{Slope}} \times 1000$$

13. TROUBLESHOOTING

Problem	Cause	Solution
No Signal for Both the Positive Control and the Sample	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 described in the protocol are followed correctly
No Signal for Only the Sample	Cells are not fixed and permeabilized sufficiently	Ensure fixation solution and permeabilizing solution are sufficiently added into cells and incubation time is enough
	The protein amount is added into well insufficiently.	Ensure extract contains a sufficient amount of proteins
High Background Present for the Blank	The well is not washed enough	Check if wash at each step is performed according to the protocol
	Overdevelopment	Decrease development time in step 11.13

14. NOTES

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