ab115051 – Histone H3 (tri-methyl K27) Assay Kit

Instructions for Use

For the measurement of global H3K27me3

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

Version 1 Last Updated 5 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. G9a and polycomb group enzyme such as EZH2 are histone methyltransferases that catalyze methylation of histone H3 at lysine 27 (H3K27) in mammalian cells. Tri-methylation of H3K27 is a facultative heterochromatin mark which promotes the recruitment of polycomb group proteins for gene silencing. Increased global H3K27 tri-methylation is also found to be involved in some pathological processes such as cancer progress. There are only a couple of methods, such as western blot, used for measuring histone H3K27 tri-methylation. The methods available so far are time consuming and labor intensive, or have low throughput.

Abcam's Histone H3 (tri methyl K27) Assay Kit addresses these problems by using a unique procedure to measure global tri-methylation of histone H3K27.

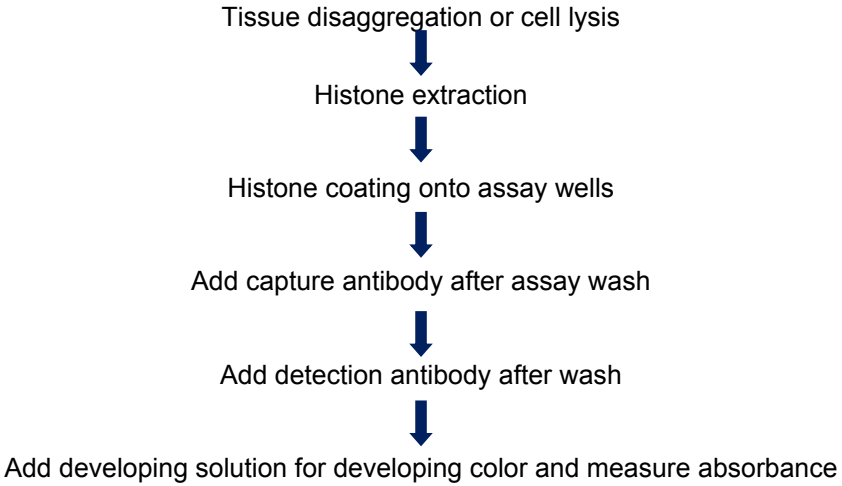
This kit has the following features:

- Quick and efficient procedure, which can be finished within 5 hours
- Innovative colorimetric assay without the need for radioactivity, electrophoresis, or chromatography
- Strip microplate format makes the assay flexible: manual or high throughput
- Simple, reliable, and consistent assay conditions

Abcam's Histone H3 (tri methyl K27) Assay Kit is designed for measuring global histone H3K27 tri-methylation. In an assay with this kit, the histone proteins are stably spotted on the strip wells. The H3K27me3 can be

recognized with a high-affinity 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.

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 Lysis Buffer	5 mL	10 mL	RT
Extraction Buffer	8 mL	16 mL	RT
10X Wash Buffer	14 mL	28 mL	4°C
Histone Buffer	0.5 mL	1 mL	RT
Blocking Buffer	10 mL	20 mL	4°C
Antibody Buffer	6 mL	12 mL	RT
Capture Antibody, 100 µg/mL	25 µL	50 µL	4°C
Detection Antibody, 400 µg/mL	10 µL	20 µL	-20°C
Developing Solution	5 mL	10 mL	4°C
Stop Solution	3 mL	6 mL	RT
H3K27me3 Control, 60 µg/mL	10 µL	20 µL	-20°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:

- Orbital shaker
- Pipettes and pipette tips
- Microplate reader
- 1.5 mL microcentrifuge tubes
- 60 or 100 mm plate
- Dounce homogenizer
- 100% TCA solution
- Glycerol
- Acetone
- 5% HCl
- Distilled water

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

10.1 Nucleic Extraction Preparation – For Tissue Samples

10.1.1 Place the tissue sample into a 60 or 100 mm plate. Remove unwanted tissue such as fat and necrotic material from the sample. Weigh the sample and cut it into small pieces (1-2 mm^3) with a scalpel or scissors.

10.1.2 Transfer tissue pieces to a Dounce homogeniser. Dilute 10X Lysis Buffer with distilled water at 1:10 ratio to make 1X Lysis Buffer. Add 1 mL of 1X Lysis Buffer per every 200 mg of tissue and disaggregate tissue pieces by 10-30 strokes.

10.1.3 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.

10.2 Nucleic Extraction Preparation – For Adherent Cells

10.2.1 Cells (treated or untreated) are grown to 70-80% confluency, then trypsinized and collected into a 15 mL conical tube. Count cells in a hemacytometer.

10.2.2 Centrifuge the cells at 1000 rpm for 5 minutes and discard the supernatant. Wash cells with 10 mL of PBS once by centrifugation at 1000 rpm for 5 minutes. Discard the supernatant.

10.2.3 Add 1X Lysis Buffer to re-suspend cell pellet (200 µL/1 x 10^6 cells). Transfer cell suspension to a 1.5 mL vial and incubate on ice for 5 minutes and vortex occasionally.

10.2.4 Pellet cell debris by centrifuging at 12,000 rpm for 30 seconds.

10.3 Nucleic Extraction Preparation – For Suspension Cells

10.3.1 Collect cells (treated or untreated) into a 15 mL conical tube. (1-2 x 10^6 cells are required for each reaction). Count cells in a hemacytometer.

10.3.2 Centrifuge the cells at 1000 rpm for 5 minutes and discard the supernatant. Wash cells with 10 mL of PBS once by centrifugation at 1 rpm for 10 minutes at 1000 rpm for 5 minutes. Discard the supernatant.

10.3.3 Add 1X Lysis Buffer to re-suspend cell pellet (100 µL/1 x 106 cells). Transfer cell suspension to a 1.5 mL vial and incubate on ice for 5 minutes. And vortex occasionally. Pellet cell debris by centrifuging at 12,000 rpm for 30 seconds.

10.4 Histone Extraction

10.4.1 Add glycerol to the Extraction Buffer at a 1:10 ratio (e.g. add 100 µL of glycerol to 900 µL of Extraction Buffer) to prepare the Extraction Buffer/Glycerol solution. Add 1X Lysis Buffer to cell debris (10 µL/1 x 106 cells or 40 mg of tissue), followed by adding 3 volumes of the Extraction Buffer/Glycerol solution. Mix by vortex and incubate on ice for 5 minutes.

10.4.2 Pellet nucleic debris by centrifuging at 12,000 rpm for 5 minutes at 4°C. Transfer the supernatant to a 1.5 mL vial.

10.4.3 Add 100% trichloroethanoic acid (TCA) to the supernatant at a 1:4 ratio (ex: add 100 µL of TCA to 300 µL of supernatant; final concentration of TCA should be 25%). Incubate on ice for 30 minutes.

10.4.4 Collect the precipitate by centrifuging at 12,000 rpm for 10 minutes at 4°C.

10.4.5 Remove supernatant and add 1 mL of acetone containing 0.1% HCl to precipitate. Mix and incubate on ice for 1 minute.

10.4.6 Collect the pellet by centrifuging at 12,000 rpm for 2 minutes at 4°C. Wash the pellet with 1 mL of acetone. Allow 1 minute on ice for wash.

10.4.7 Collect the pellet by centrifuging at 12,000 rpm for 2 minutes at 4°C. Remove the supernatant as much as possible and air dry the pellet for 5 minutes.

10.4.8 Add distilled water to dissolve pellet (10 µL of water per amount of pellet extracted from 1 x 10^6 cells or 40 mg of tissue) and measure histone protein concentration. The histone extract can be used immediately or stored at -20°C.

11. ASSAY PROCEDURE

11.1 Histone H3K27 Tri-Methylation Detection

11.1.1 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). Dilute the 10X Wash Buffer with distilled water (pH 7.2-7.5) at a 1:10 ratio to make 1X Wash Buffer.

11.1.2 Adjust protein concentration to 200 ng/µL or 400 ng/µL with Histone Buffer and add 5 µL (1-2 µg) of the protein solution into the central area of each well. Spread out the solution over the bottom of the strip well by pipetting the solution up and down several times, and incubate at 37°C (with no humidity) for 60-90 minutes to evaporate the solution and dry the wells. For the blank, add 5 µL of Histone Buffer to the wells. For the positive control, dilute the H3K27me3 Control to 2-30 ng/µL with Histone Buffer, and then add 5 µL (10-150 ng) of the diluted H3K27me3 Control solution to the wells.

11.1.3 Add 150 µL of Blocking Buffer to the dried wells and incubate at 37°C for 30-45 minutes.

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

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

11.1.6 Aspirate and wash the wells with 150 µL of the 1X Wash Buffer four times.

11.1.7 Dilute the Detection Antibody (at a 1:1000 ratio) to 0.4 µg/mL with Antibody Buffer. Add 50 µL of the Diluted Detection Antibody to the wells and incubate at room temperature for 30 minutes.

11.1.8 Aspirate and wash the wells with 150 µL of 1X Wash Buffer five times.

11.1.9 Add 100 µL of the 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 standard wells (blue).

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

11.1.11 Calculate % H3K27 tri-methylation using the formulae provided in Section 12 – Data Analysis.

12. ANALYSIS

Calculate the % H3K27 tri-methylation using the following formula:

$$\text{Methylation \%} = \frac{\text{Sample OD} - \text{Blank OD}}{\text{Untreated Control OD} - \text{Blank OD}} \times 100\%$$

For an 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:

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

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 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 Positive Control	The positive control is added insufficiently	Ensure a sufficient amount of control is added to the well
	The positive control is degraded due to incorrect storage	Follow the guidance in the protocol for storage of positive control
No Signal for Only the Sample	The protein sample is not properly extracted	Follow the protocol instructions for the histone protein extraction
	The protein amount is added into well insufficiently	Ensure extract contains a sufficient amount of protein
	Protein extracts are incorrectly stored or were stored for a long time	Ensure the protein extracts are stored at -80°C for no more than 8 weeks
High Background Present for the Blank	The well is not washed enough	Check if wash at each step is performed according to the protocol

	Contaminated by the positive control	Ensure the well is not contaminated from adding the control protein or from using control protein contaminated tips
	Overdevelopment	Decrease development time at step 11.1.9

14. NOTES

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp