Catalog	Kit Size
TBS32124-48	48 assays
TBS32124-96	96 assays

INTRODUCTION

Tri-Methyl Histone H3K4 refers to the trimethylation of the lysine 4 residue on histone H3. H3K4 tri-methylation has been viewed as a signature mark of highly transcribed genes, which is placed exclusively in the 5'- region downstream of the promoter. Increased global H3K4 tri-methylation is also found to be involved in some pathological processes such as cancer progression. Therefore, quantitative detection of global tri-methyl histone H3K4 would provide useful information for better understanding epigenetic regulation of gene activation, and for developing HMT-targeted drugs.

The Global Tri-Methyl H3K4 Quantification Kit is designed to measure global histone H3K4 tri-methylation in various mammalian cells, including human and mouse cells, as well as fresh and frozen tissues, cultured adherent cells, and suspension cells. This assay is simple, accurate, and fast. The measurement can be completed in 2.5 hours, with a detection range of 20 ng to 5 µg per well.

Alternative Name: Trimethylation of Histone H3 at Lysine 4; H3K4me3; H3-K4

PRINCIPLE OF THE ASSAY

In an assay with this kit, the tri-methylated histone H3 at lysine 4 is captured to the strip wells coated with an anti-trimethyl H3K4 antibody. The captured tri-methylated histone H3K4 can then be detected with a labeled detection antibody, followed by a color development reagent. The ratio of tri-methylated H3K4 is proportional to the intensity of absorbance. The absolute amount of tri-methylated H3K4 can be quantitated by comparing to the standard control.

KIT CONTENT AND STORAGE CONDITIONS

PART	48 ASSAYS	96 ASSAYS	STORAGE OF OPENED/ RECONSTITUTED
Detection Antibody	5 µL of histone H3K4 antibody(1000x).	10 µL of histone H3K4 antibody(1000x).	–20°C
Histone H3K4 Standard	10 µL of histone H3K4 control (100ng/µL).	20 µL of histone H3K4 control (100ng/µL).	–20°C
Stop Solution	3 mL of 2 N sulfuric acid.	6 mL of 2 N sulfuric acid.	Room temperature away from light
Wash Buffer	10 mL of concentrated solution (10x).	20 mL of concentrated solution (10x).	May be stored for up to 6 months at 2-8 °C.
Antibody Buffer	6 ml	12 ml	
Color Developer	5 ml	10 ml	
8-Well Sample Strips (with Frame) Precoated with H3K4 antibody	6	12	

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PRECAUTIONS

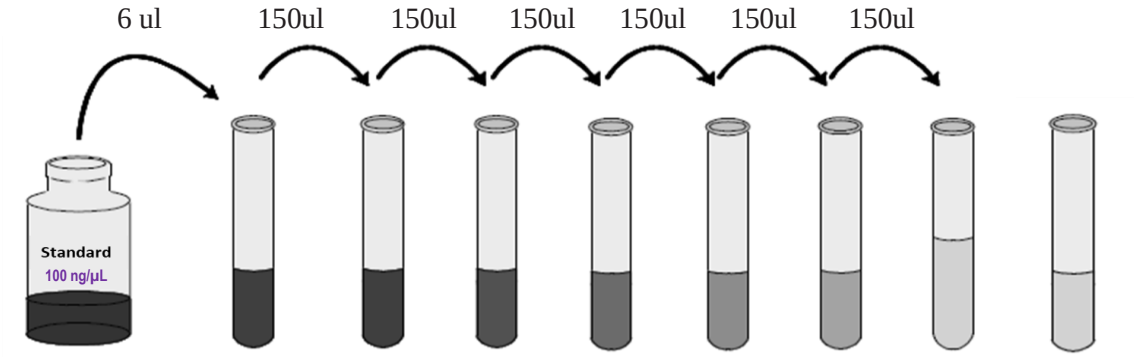
Wear protective gloves, clothing, eyes, and face protection. Wash hands thoroughly after handling.

PROTOCOL

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).
2. Dilute 10x **Wash Buffer** with distilled water at a 1:9 ratio (ex: 1 ml of 10x **Wash Buffer** + 9 ml of water) to 1x Wash Buffer.
3. Add 50 µl of **Sample** into sample well (sample protein amount: 50-200 ng of the histone extract).
4. Standard Preparation: Label test tubes as #1 through #8. Pipet 294 µL of 1x Antibody Buffer into tube #1, and 150 µL into tubes #2 to #7 **as diagram below (Fig.2).**

Add 6 µL of the Standard Stock (100 ng/µL) to tube #1, and mix. Then, make 2x serial dilutions of the standard using the tube #1 standard solution from tube #2 through #7 with sequential transfer of 150 µL to the next concentration. Mix each tube thoroughly before the next transfer.
Add 50 µL of each standard concentration into the indicated standard well. The standard amount of 50 µL in tubes 1 through 7 will be 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 ng/well. Tube# 8 is Standard 0.

Fig.2 Diagram for Tri-Methyl Histone H3K4 standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Antibody Buffer (µL)	294	150	150	150	150	150	150	150
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	6	150	150	150	150	150	150	0
Amount (ng/well)	100	50	25	12.5	6.25	3.13	1.56	0

5. Incubate the plate at room temperature for 2 hours.
6. Aspirate and wash the wells with 150 µl of 1x **Wash Buffer** three times.
7. Dilute **Detection Antibody** (at a 1:1000 ratio) to working concentration 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).
8. Aspirate and wash the wells with 150 µl of 1x **Wash Buffer** for six times.
9. Add 100 µl of **Color Developer** into 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).
10. Add 50 µl of **Stop Solution** to each well to stop enzyme reaction when the color in the standard wells containing the higher concentrations of standard control turns medium blue. The color should change to yellow and absorbance can be read on a microplate reader at 450 nm within 2-15 minutes.
11. Calculate % histone H3K4 tri-methylation:

$$Tri-methylation\% = \frac{OD\ (treated\ (tested)\ sample - blank)}{OD\ (untreated\ (control)\ sample - blank)} \times 100\%$$

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

Calculate the amount of tri-methylated H3K4 using the following formula:

$$Amount\ (ng/mg\ protein) = \frac{OD\ (sample - blank)}{Protein\ (\mu g) \times slope} \times 1000$$

HISTONE EXTRACTION PROTOCOL

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 or PBS containing 0.5% Triton X 100 or 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.
2. Resuspend cell/tissue pellet in 3 volumes (approx. 200 µl/10^7 cells or 200 mg of tissue) of extraction buffer (0.5N HCl + 10% glycerol) and incubate on ice for 30 minutes.
3. Centrifuge at 12,000 rpm for 5 minutes at 4°C and remove the supernatant fraction to a new vial.
4. Add 8 volumes (approx. 0.6 ml/10^7 cells or 200 mg of tissue) of acetone and leave at –20°C overnight.
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 of tissue).
6. Quantify the protein concentration. Aliquot the extract and store the extract at –20°C or –80°C.

RELATIVE PRODUCTS

Total Histone H3 Acetylation Detection (Colorimetric) (TBS32120)
Global Acetyl Histone H3K9 Quantification (Colorimetric) (TBS32121)
Total Histone H3 Acetylation Detection (Fluorometric) (TBS32122)
Global Histone H3K4 Methylation Assay Kit (TBS32123)
Histone H3 Modification Multiplex Assay Kit (Colorimetric) (TBS32125)
HDAC Activity Fluorometric Assay (TBS2060)
HDAC Activity Colorimetric Assay (TBS2065)
Adenosyl homocysteinase (AHCY) Fluorometric Assay (TBS2056)
NNMT Activity Fluorometric Assay (TBS2098)
Adenosylhomocysteine (AHCY) Inhibitor Screening Assay (Fluorometric) (TBS2099)
NNMT Inhibitor Screening Assay (TBS2097)
Chromatin Immunoprecipitation (ChIP) Assay (TBS8050)

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