Catalog	Kit Size
TBS32123-48	48 assays
TBS32123-96	96 assays

INTRODUCTION

H3K4 methylation refers to the addition of methyl groups to the lysine 4 residue of histone H3, a process that can occur in three states: mono-, di-, and trimethylation (H3K4me1, H3K4me2, and H3K4me3). This modification is associated with transcriptional activation and is typically found at the promoter and enhancer regions of actively transcribed genes. H3K4 methylation is a conserved chromatin modification across various organisms, from yeast to humans. H3K4 methylation plays a critical role in gene expression regulation, chromatin structure, and various biological processes, including development and differentiation.

The Global Histone H3K4 Methylation Assay is designed to measure H3-K4 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 5 hours, with a detection range of 2 µg to 5 ng per well.

Alternative Name: histone H3 lysine 4 methylation, H3K4me

PRINCIPLE OF THE ASSAY

In an assay with this kit, the histone proteins are stably spotted on the strip wells. A high-affinity antibody specifically detects methylated histone H3-K4. The level or proportion of methylated H3-K4 is measured using an HRP-conjugated secondary antibody and a color development system, with the intensity of the color directly correlating to the amount of methylation.

KIT CONTENT AND STORAGE CONDITIONS

PART	48 ASSAYS	96 ASSAYS	STORAGE OF OPENED/ RECONSTITUTED
Detection Antibody	10 µl of histone H3K4 antibody(1000x).	20 µl of histone H3K4antibody(1000x).	–20°C
Methylated H3-K4 Control	10 µL of histone H3K4 control.	20 µL of histone H3K4 control.	–20°C
Wash Buffer	14 ml of concentrated solution (10x).	28 ml of concentrated solution (10x).	May be stored for up to 6 months at 2-8 °C.
Blocking Buffer	10 ml	20 ml	
Capture Antibody	25 µl	50 µl	
Developing Solution	5 ml	10 ml	
8-Well Sample Strips (with Frame) Precoated with H3K4 antibody	6	12	
Antibody Buffer	6 ml	12 ml	Room Temperature
10X Lysis Buffer	5 ml	10 ml	
Extraction Buffer	8 ml	16 ml	
Histone Buffer	0.5 ml	1 ml	
Stop Solution	3 ml	6 ml	

Store the unopened kit at 2-8 ℃. 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 (pH 7.2-7.5) at a 1:10 ratio to 1x wash buffer (ex: 1 ml of 10x **Wash**

Buffer + 9 ml of distilled water).

3. 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 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 **Methylated H3-K4 Control** to 2-30 ng/µl with **Histone Buffer**, and then add 5 µl (10-150 ng) of the diluted **Methylated H3-K4 Control** solution to the wells.
4. Add 150 µl of **Blocking Buffer** to the dried wells and incubate at 37˚C for 30-45 minutes.
5. Aspirate and wash the wells with 150 µl of 1x **Wash Buffer** for three times.
6. Dilute **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).
7. Aspirate and wash the wells with 150 µl of 1x **Wash Buffer** four times.
8. Dilute **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.
9. Aspirate and wash the wells with 150 µl of 1x **Wash Buffer** five times.
10. 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. Add 50 µl of **Stop Solution** to the wells and read absorbance on a microplate reader at 450 nm.
12. Calculate % H3-K4 methylation:

$$Methylation\,\% = \frac{OD\,(sample - blank)}{OD\,(untreated\ control - blank)} \times 100\%$$

For accurate calculation, plot OD value versus amount of **Methylated H3-K4 Control** and determine the slope as delta OD/ng.

Calculate the amount of methylated H3-K4 using the following formula:

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

NUCLEIC EXTRACTION

For Tissue Samples:

1. Dilute 10X **Lysis Buffer** with distilled water at a 1:10 ratio (ex: 1 ml of 10X **Lysis Buffer** + 9 ml of distilled water) to make 1x lysis buffer.
2. 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.
3. Transfer tissue pieces to a Dounce homogenizer. Add 1 ml of the diluted **Lysis Buffer** per every 200 mg of tissue and disaggregate tissue pieces by 10-30 strokes.
4. Transfer homogenized mixture to a 15 ml conical tube and centrifuge at 3000 rpm for 5 minutes at 4°C. If the 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 Adherent Cells:

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

For Suspension Cells:

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.
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 10 minutes at 4°C for 5 minutes. Discard the supernatant.
3. Add 1x **Lysis Buffer** to re-suspend cell pellet (100 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. Pellet cell debris by centrifuging at 12,000 rpm for 30 seconds.

HISTONE EXTRACTION PROTOCOL

1. Add glycerol to **Extraction Buffer** at a 1:10 ratio (ex: add 100 µl of glycerol to 900 µl of **Extraction Buffer**) to prepare the **Extraction Buffer /Glycerol solution**. Add the diluted **Extraction Buffer** to cell debris (10 µl/1 x 10^6 cells or 40 mg of tissue), followed by adding 3 volumes of **Extraction Buffer/Glycerol solution**. Mix by vortex and incubate on ice for 5 minutes.
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.
3. Add 100% TCA (**Trichloroacetic Acid**) solution 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.
4. Collect the precipitate by centrifuging at 12,000 rpm for 10 minutes at 4°C.
5. Remove supernatant and add 1 ml of acetone containing 0.1% HCl to precipitate. Mix and incubate on ice for 1 minute.
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.
7. Collect the pellet by centrifuging at 12,000 rpm for 2 minutes at 4°C. Remove supernatant as much as possible and air dry the pellet for 5 minutes.
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 –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 Tri-Methyl Histone H3K4 Quantification Kit (Colorimetric) (TBS32124)
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)

For research use only.