Catalog	Kit Size
TBS32122	96 assays

INTRODUCTION

Histone H3 acetylation involves the addition of acetyl groups to lysine residues on histone proteins, which neutralizes their positive charge and relaxes chromatin structure, facilitating gene transcription, which are associated with DNA in chromatin. Histone acetylation is regulated by histone acetyltransferases (HATs), which add acetyl groups, and histone deacetylases (HDACs), which remove them. Histone H3 acetylation is tightly involved in cell cycle regulation, cell proliferation, and apoptosis. Disruption of histone acetylation balance in cancer cells, linked to abnormal transcription factor binding, promotes tumorigenesis, cancer progression, and metastasis.

The Total Histone H3 Acetylation Detection Assay is designed to measure histone H3 acetylation 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, user-friendly, accurate, and fast. The measurement can be completed in 2.5 hours, with a detection range of 2 µg to 5 ng per well.

Alternative Name: H3ac

PRINCIPLE OF THE ASSAY

In this assay, an acetylated histone H3 binds to the capture antibody against a specific acetylated histone H3 which is immobilized within the strip wells to make an antibody-antigen complex. Following this step, the bound acetyl histone H3 is detected using a secondary antibody conjugated to a detectable label. A subsequent reaction with a fluorescent development reagent produces a measurable signal, where the intensity of fluorescence is directly proportional to the relative amount of acetylated histone H3 present in the sample. The absolute amount of acetyl histone H3 is quantified from a control standard with known concentrations, ensuring precise measurement.

KIT CONTENT AND STORAGE CONDITIONS

PART	96 ASSAYS	STORAGE OF OPENED/ RECONSTITUTED
Detection Antibody	10 µL of histone H3 antibody(1000x).	–20°C
Histone H3 Standard	20 µL of histone H3 control.	–20°C
Fluoro Developer	24 µl	–20°C
Wash Buffer	20 mL of concentrated solution (10x).	May be stored for up to 6 months at 2-8 °C.
Antibody Buffer	12 ml	
Fluoro Enhancer	24 µl	
Fluoro Dilution	8 mL	
8-Well Sample Strips (with Frame) Precoated with H3 antibody	12	

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

PRECAUTIONS

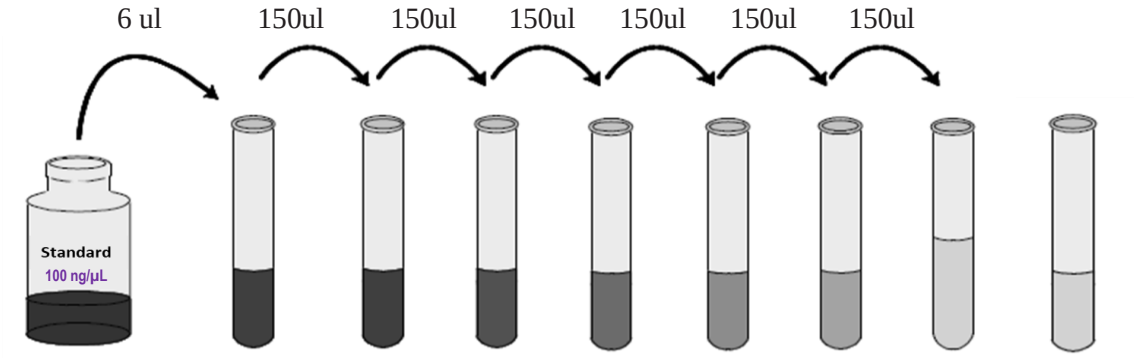
Wear protective gloves, clothing, eyes, and face protection. Wash hands thoroughly after handling.

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 to 1x wash buffer (ex: 1 ml of 10x **Wash Buffer** + 9 ml of water).
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 Control (100 ng/µL) to tube #1, and mix. **Then,** make 2x serial dilutions of the standard using 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 Acetyl Histone H3K9 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. Aspirate and wash the wells with 150 µl of 1x **Wash Buffer** for three times.
6. Dilute **Detection Antibody** (at a 1:1000 ratio) to working detection 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).
7. Aspirate and wash the wells with 150 µl of 1x **Wash Buffer** six times.
8. Prepare the **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 the **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 $530_{EX}/590_{EM}$ 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 $530_{EX}/590_{EM}$ nm
9. Calculate % histone H3 acetylation:

$$Acetylation\ \% = \frac{RFU\ (treated\ (tested)\ sample - blank)}{RFU\ (untreated\ (control)\ sample - blank)} \times 100\%$$

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

Calculate the amount of acetyl H3 using the following formula:

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

* Histone extract amount added into the sample well at step 3.

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)
Global Histone H3K4 Methylation Assay Kit (TBS32123)
Global Tri-Methyl Histone H3K4 Quantification Kit (Colorimetric) (TBS32124)
Histone H3 Modification Multiplex Assay Kit (Colorimetric) (TBS32125)

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