Global Histone H3 Phosphorylation (Ser10) Assay Kit (Colorimetric)

Catalog Number KA0703

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

The Global Histone H3 Phosphorylation (Ser10) Assay Kit (Colorimetric) provides a tool for measuring global phospho histone H3 (Ser10).

Features:

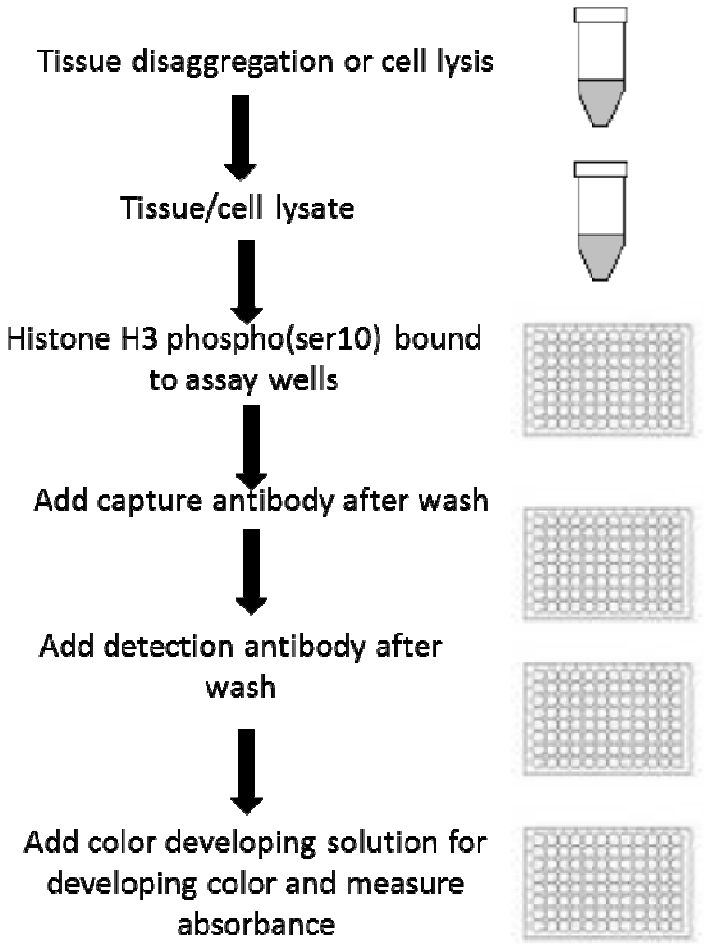
- ✓ Quick and efficient procedure, which can be finished within 3 hours.
- ✓ Innovative colorimetric assay with no need for radioactivity, electrophoresis, or chromatography.
- ✓ Specifically captures phospho histone H3 (Ser10) with the detection limit as low as 2 ng/well.
- ✓ The control is conveniently included for quantification of the amount of phosphorylated histone H3 (Ser10).
- ✓ Strip microplate format makes the assay flexible: manual or high throughput.
- ✓ Simple, reliable, and consistent assay conditions.

Background

The phosphorylation of histone H3 at serine 10 is conserved through eukaryotes, and an increase in phosphorylation has been shown to correlate with gene activation and cell growth. In vitro studies have shown that phosphorylation of histone H3 at Ser10 is coupled to acetylation at the nearby Lysine-14 residue. Histone H3 phosphorylation at Ser10 is also negatively impacted by histone methylation at lysine 9. It was observed that histone H3 phosphorylation at Ser10 is regulated by the cell cycle and has been used as mitotic marker. H3 phosphorylation (Ser10) is critical for neoplastic cell transformation. Several protein kinases, including aurora B, PPI, and PKC, are responsible for histone H3 phosphorylation at Ser10. Inhibition or activation of these protein kinases can cause the change in intracellular histone H3 phosphorylation at Ser10. Detection in the change of histone H3 phosphorylation at Ser10 associated with cell cycle, apoptosis, and inhibitor or activator treatment, would provide useful information for better understanding the pathological process of some diseases and for protein kinase-targeted drug development.

Principle of the Assay

The Global Histone H3 Phosphorylation (Ser10) Assay Kit (Colorimetric) is designed for measuring global histone H3 phosphorylation at Ser10. In an assay with this kit, the phosphorylated histone H3 at Ser10 is captured to the strip wells coated with an anti-phospho histone H3 (Ser10) antibody. The captured phospho histone H3 (Ser10) can then be detected with a labeled detection antibody followed by a color development reagent. The ratio of phospho histone H3 (Ser10) is proportional to the intensity of absorbance. The absolute amount of phospho histone H3 (Ser10) can be quantitated by comparing to the standard control.


Tissue disaggregation or cell lysis
Tissue/cell lysate
Histone H3 phospho(ser10) bound to assay wells
Add capture antibody after wash
Add detection antibody after wash
Add color developing solution for developing color and measure absorbance

General Information

Materials Supplied

List of component

Component	Amount
C1 (10X wash buffer)	20 ml
C2 (antibody buffer)	12 ml
C3 (detection antibody, 1 mg/ml)*	10 µl
C4 (color developer)	10 ml
C5 (stop solution)	6 ml
Standard control (100 µg/ml)*	20 µl
8 well sample strips (with frame)	9
8 well standard control strips*	3

* For maximum recovery of the products, centrifuge the original vial after thawing prior to opening the cap.

Storage Instruction

The kit is shipped in two parts, one part at ambient room temperature, and the second part on frozen ice packs at 4°C.
Upon receipt, store C3 and Standard control at - 20℃. Store all other components at 4℃ away from light. The components of the kit should be stable for 6 months when stored properly.

Note: Check if buffers C1 and C2 contain salt precipitates before using. If so, warm (at room temperature or 37℃) and shake the buffers until the salts are re-dissolved.

Materials Required but Not Supplied

- ✓ Orbital shaker
- ✓ Pipettes and pipette tips
- ✓ Reagent reservoir
- ✓ Microplate reader

Precautions for Use

- ✓ This kit has been configured for research use only and is not for diagnostic and clinical use.
- ✓ Suitable lab coat, disposable gloves, and eye protection are required when working with the kit.

Assay Protocol

Assay Procedure

1. a) Prepare histone extracts from cells/tissues treated or untreated by using your own successful method (acid extraction or high salt extraction).
   b) Preparation of histone extracts can be also performed using the attached procedure (See Appendix). Histone extracts can be used immediately or stored at –80℃ for future use.
2. Determine the number of the strip wells required. Leave these strips in the plate frame (remaining unused strips can be put back in the bag. Seal the bag tightly and store at 4℃). Dilute C1 with distilled water (pH 7.2-7.5) at the 1:9 ratios (1 ml of C1+ 9 ml of water).
3. Add 50 µl of C2 into each well. For the sample, add 1-2 µg of the histone extract into the sample wells. For standard curve, dilute standard control with C2 to 1-100 ng/µl for 5-7 points (ie: 1.5, 3, 6, 12, 25, 50, and 100 ng/µl). Add 1 µl of standard control at the different concentrations into the standard well. For the blank, do not add any nuclear extracts or standard control protein. Mix and cover the strip wells with Parafilm M and incubate at room temperature for 1-2 hours.
4. Aspirate and wash the wells with 150 µl of diluted C1 three times.
5. Dilute C3 (at 1:1000 ratio) to 1 µg/ml with C2. Add 50 µl of diluted C3 to each well and incubate at room temperature for 60 minutes on an orbital shaker (100 rpm).
6. Aspirate and wash the wells with 150 µl of diluted C1 six times.
7. Add 100 µl of C4 into the wells and incubate at room temperature for 2-10 min away from light. Monitor color development in the sample and standard wells (blue).
8. Add 50 µl of C5 to each well to stop enzyme reaction when 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.

Plate Configuration

- ✓ Strip 1-3 (for 96 assays): standard wells (labeled as SC); the standard curve can be generated with 5-8 concentration points (includes blank).
- ✓ Example amount of standard control /well: A1: 100 ng; B1: 50 ng; C1: 25 ng; D1: 12 ng; E1: 6 ng; F1: 3 ng; G1 1.5 ng; H1: 0 ng.
- ✓ Strip 4-12 (for 96 assays): sample wells (No label).
- ✓ Each sample or standard point can be assayed in the duplicates or triplicates.

Appendix

Histone Extraction protocol

1. For tissues (treated and untreated), weigh the sample and Cut the sample into small piece (1-2 mm^3) with a scalpel or scissors. Transfer tissue pieces to a Dounce homogenizer. Add TEB buffer (PBS containing 0.5% Triton X 100, 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 3,000 rpm for 5 minutes at 4℃. 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℃. Remove supernatant.

   For cells (treated and untreated). Harvest cells and pellet the cells by centrifugation at 1000 rpm for 5 min at 4℃. Resuspend cells in TEB buffer at 10^7 cells/ml and lyse cells on ice for 10 min with gentle stirring. Centrifuge at 3000 rpm for 5 min at 4℃. If total volume is less than 2 ml, transfer cell lysates to a 2 ml vial and centrifuge at 10000 rpm for 1 min at 4℃. 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 min at 4℃ and remove the supernatant fraction to new vial.
4. Add 8 volumes (approx. 0.6 ml/ 10^7 cells or 200 mg of tissue) of acetone and leave at –20℃ overnight.
5. Centrifuge at 12,000 rpm for 5 min 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℃ or –80℃.

Data Analysis

Calculation of Results

Calculate % histone H3 phospho (Ser 10):

$$\text{Phospho (Ser 10) \%} = \frac{\text{OD (treated (tested) sample – blank)}}{\text{OD (untreated (control) sample – blank)}} \times 100\%$$

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

Calculate the amount of phospho (Ser 10) using the following formula:

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

Resources

Troubleshooting

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 in the protocol is correctly followed.

No Signal or Very Weak Signal for Only the Positive Control

The amount of standard control is not added into the “standard control wells”, or is added insufficiently.	Ensure sufficient amount of control is properly added to the standard control wells.

No Signal for Only the Sample

The protein sample is not properly extracted.	Ensure the procedure and reagents are correct for the nuclear protein extraction.
The protein amount is added into well insufficiently.	Ensure extract contains enough amounts of proteins.
Protein extracts are incorrectly.	Ensure the nuclear extracts are stored at –20℃ or –80℃.

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 standard control.	Ensure the well is not contaminated by adding the control protein or by using control protein contaminated tips.
Overdevelopment.	Decrease development time in protocol step 7.

Plate Layout

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	A	B	C	D	E	F	G	H