ab115130 – Histone H3 (phospho S28) Assay Kit (Fluorometric)

Instructions for Use

For the measurement of global histone H3S28 phosphorylation using a variety of mammalian cells including fresh and frozen tissues, and cultured adherent and suspension cells

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

Version 1 Last Updated 3 September 2014

Table of Contents

INTRODUCTION

1. BACKGROUND 2
2. ASSAY SUMMARY 3

GENERAL INFORMATION

3. PRECAUTIONS 4
4. STORAGE AND STABILITY 4
5. MATERIALS SUPPLIED 5
6. MATERIALS REQUIRED, NOT SUPPLIED 5
7. LIMITATIONS 6
8. TECHNICAL HINTS 6

ASSAY PREPARATION

9. REAGENT PREPARATION 7
10. SAMPLE PREPARATION 7
11. PLATE PREPARATION 8

ASSAY PROCEDURE

12. ASSAY PROCEDURE 9

DATA ANALYSIS

13. ANALYSIS 10

RESOURCES

14. TROUBLESHOOTING 11
15. NOTES 12

1. BACKGROUND

The phosphorylation of histone H3 at serine 28 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 both ser10 and ser28 is coupled to dynamic acetylation of histone H3, where H3 phosphorylated at serine 28 had a higher steady state of acetylation than that of H3 phosphorylated at serine 10. It was observed that histone H3 phosphorylation at ser28 is regulated by the cell cycle and has been used as a mitotic marker. As with phosphorylated ser10, H3 phosphorylation at ser28 also plays an important role for neoplastic cell transformation. Several protein kinases including aurora B, PPI, and PKC are responsible for histone H3 phosphorylation at ser28. Inhibition or activation of these protein kinases can cause a change in intracellular histone H3 phosphorylation at ser28. Detection of the change in histone H3 phosphorylation at ser28 associated with the cell cycle, apoptosis, and inhibitor or activator treatment, would provide useful information for better understanding the pathological processes of some diseases and for protein kinase-targeted drug development.

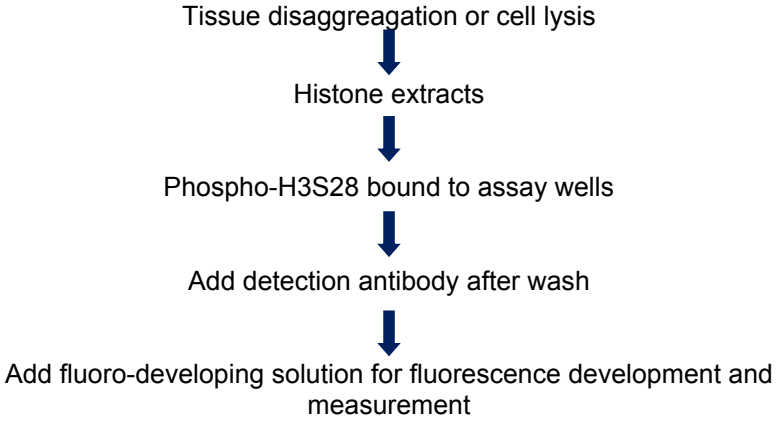
ab115130 provides a tool for measuring global phospho histone H3 (ser28).

This kit has the following features:

- Quick and efficient procedure, which can be finished within 3 hours
- Innovative fluorometric assay without the need for radioactivity, electrophoresis, or chromatography
- Specifically captures phospho histone H3 (ser28) with the detection limit as low as 0.5 ng/well
- The control is conveniently included for the quantification of phosphorylated histone H3 (ser28)
- Strip microplate format makes the assay flexible: manual or high throughput
- Simple, reliable, and consistent assay conditions

Abcam's Histone H3 (phospho S28) Assay Kit (Fluorometric) is designed for measuring global histone H3 phosphorylation at ser28. In an assay with this kit, the phosphorylated histone H3 at ser28 is captured to the strip wells coated with an anti-phospho histone H3 (ser28) antibody. The captured phospho histone H3 (ser28) can then be detected with a labeled detection antibody followed by a fluoro development reagent. The ratio of phospho histone H3 (ser28) is proportional to the intensity of fluorescence. The absolute amount of phospho histone H3 (ser28) can be quantitated by comparing to the standard control.

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 and away from light 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 and Antibody Buffer contain 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 Wash Buffer	10 mL	20 mL	4°C
Antibody Buffer	6 mL	12 mL	4°C
Detection Antibody, 1 mg/mL	5 µL	10 µL	-20°C
Fluoro Developer	12 µL	24 µL	-20°C
Fluoro Enhancer	12 µL	24 µL	4°C
Fluoro Dilution	4 mL	8 mL	4°C
Standard Control, 100 µg/mL	10 µL	20 µL	-20°C
8 Well Assay Strip (with Frame)	4	9	4°C
8 Well Standard Control Strips*	2	3	4°C

*Green Ringed Wells.

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
- Reagent reservoir
- Fluorescence Microplate reader
- 15 mL conical tube
- 1.5 mL microcentrifuge tubes

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

9.1 **1X Wash Buffer**

Dilute 10X Wash Buffer with distilled water (pH 7.2-7.5) at a 1:10 ratio (1 mL of 10X Wash Buffer + 9 mL of water).This diluted 1X Wash Buffer can now be stored at 4°C for up to six months.

9.2 **Detection Antibody**

Dilute the Detection Antibody (at 1:1000 ratio) to 1 µg/mL with Antibody Buffer.

9.3 **Fluoro Development Sloution**

Adding 1 µL of Fluoro Developer and 1 µL of Fluoro Enhancer into each 400 µL of Fluoro Dilution store away from light.

10. SAMPLE PREPARATION

Prepare histone extracts from cells/tissues treated or untreated by using your own successful method (acid extraction or high salt extraction). For your convenience and the best results, Abcam offers the Histone Extraction Kit (ab113476) optimized for use in Abcam's modified histone quantification series. Alternatively, preparation of histone extracts can also be performed using the procedure below:

10.1 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 (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 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.

10.2 Resuspend cell/tissue pellet in 3 volumes (approx. 200 µL/10^7 cells or 200 mg tissues) of extraction buffer (0.5N HCl + 10% glycerol) and incubate on ice for 30 minutes.

10.3 Centrifuge at 12,000 rpm for 5 minutes at 4°C and remove the supernatant fraction to a new vial.

10.4 Add 8 volumes (approx. 0.6 mL/10^7 cells or 200 mg tissues) of acetone and leave at -20°C overnight.

10.5 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 tissues).

10.6 Quantify the protein concentration. Aliquot the extract and store the extract at -20°C or -80°C.

Histone extracts can be used immediately or stored at -80°C for future use.

11. PLATE PREPARATION

- Strip 1-3 (for 96 assays) or strip 1-2 (for 48 assays) – standard wells (green colored trim); 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) or strip 3-6 (for 48 assays) – sample wells (No label).
- Each sample or standard point can be assayed in duplicates or triplicates.

12. ASSAY PROCEDURE

12.1 Predetermine the number of strip wells required. Remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

12.2 Add 50 µL of the Antibody Buffer into each well. For the sample, add 1-2 µg of the histone extract into the sample wells. For the standard curve, dilute the Standard Control with the Antibody Buffer to 1 – 100 ng/µL at 5-7 points (e.g., 1.5, 3, 6, 12, 25, 50, and 100 ng/µL). Add 1 µL of Standard Control at the different concentrations into the standard wells (ringed in green). 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.

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

12.4 Add 50 µL of Diluted Detection Antibody to each well and incubate at room temperature for 60 minutes on an orbital shaker (100 rpm).

12.5 Aspirate and wash the wells with 150 µL of 1X Wash Buffer six times.

12.6 Add 50 µL of 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 Ex/Em = 530/590 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 Ex/Em = 530/590 nm.*

12.7 Calculate % histone H3S28 phosphorylation using the formulae provided in Section 13 – Data Analysis.

13. ANALYSIS

Calculate the % Histone H3S28 phosphorylation using the following formula:

$$\text{Phosphorylation \%} = \frac{\text{Treated (Tested) Sample RFU} - \text{Blank RFU}}{\text{Untreated (Control) Sample RFU} - \text{Blank RFU}} \times 100\%$$

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

Calculate the amount of phosphorylated H3S28 using the following formula:

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

*Histone extract amount added into the sample well at step 12.2.

14. TROUBLESHOOTING

Problem	Cause	Solution
No Signal for Both the Standard 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 Standard Control	The amount of Standard control is not added into the “standard control wells or is added insufficiently	Ensure a sufficient amount of control is added to the well
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 a sufficient amount of protein
	Protein extracts are incorrectly stored	Ensure the protein extracts are stored at -20°C or -80°C
High Background Present for the Blank	The well is not washed sufficiently	Check if wash at each step is performed according to the protocol
	Contaminated by the Standard control	Ensure the well is not contaminated from adding the control protein or by using control protein contaminated tips
	Overdevelopment	Decrease development time in step 12.6

15. 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: 108008523689 (中國聯通)

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

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

Copyright © 2014 Abcam, All Rights Reserved. The Abcam logo is a registered trademark.
All information / detail is correct at time of going to print.