EpiQuik™ In Situ Histone H3 Phosphorylation (Ser10) Assay Kit

Base Catalog # P-7001

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik™ In Situ* Histone H3 Phosphorylation (Ser10) Assay Kit is suitable for specifically measuring histone H3 phosphorylation (ser10) *in situ* using cultured adherent cells.

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KIT CONTENTS

Components	96 assays P-7001-96
PA1 (10X Wash Buffer)	30 ml
PA2 (Permeabilizing Buffer)	30 ml
PA3 (Blocking Buffer)	20 ml
PA4 (Antibody Buffer)	15 ml
PA5 (Capture Antibody, 1000 µg/ml)*	6 µl
PA6 (Detection Antibody, 200 µg/ml)*	20 µl
PA7 (Developing Solution)	12 ml
PA8 (Stop Solution)	6 ml
30% H_2O_2 Solution	0.5 ml
Phospho $H3^{Ser10}$ Control (20 µg/ml)	15 µl
8-Well Control Strips	2
Microplates	1

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

SHIPPING & STORAGE

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: (1) Store **PA6** and **Phospho $H3^{Ser10}$ Control** at –20°C; (2) Store **PA8** at room temperature away from light; (3) Store **all other components** at 4°C away from light. The kit is stable for up to 6 months from the shipment date, when stored properly.

*Note: Check if buffers, **PA1** and **PA4**, contain salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffers until the salts are re-dissolved.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Pipettes and pipette tips
- ☐ Microplate reader
- ☐ 15 ml conical tube
- ☐ 1.5 ml microcentrifuge tubes
- ☐ 37% formaldehyde
- ☐ PBS

GENERAL PRODUCT INFORMATION

Usage Limitation: The *EpiQuik™ In Situ* Histone H3 Phosphorylation (Ser10) Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

Quality Control: EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Intellectual Property: *EpiQuik™* is a trademark of EpigenTek Group Inc.

A BRIEF OVERVIEW

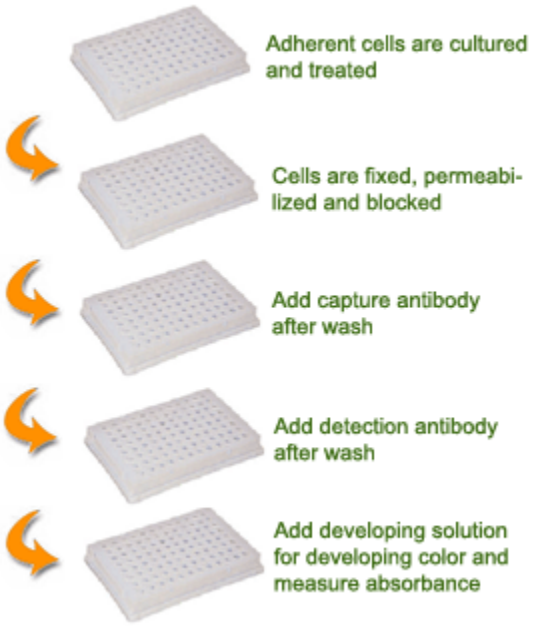
The phosphorylation of histone H3 at serine 10 is conserved through eukaryotes, and an increase in phosphorylation has been shown to correlate with gene activation and cell growth. *In vitro* studies have shown that *phosphorylation* of histone H3 at ser10 is coupled to acetylation at the nearby Lysine-14 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 a 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 the cell cycle, apoptosis, inhibitor or activator treatment, would provide useful information for better understanding the pathological process of some diseases and for protein kinase-targeted drug development. The *EpiQuik™ In Situ* Histone H3 Phosphorylation (Ser10) Assay Kit provides a tool for measuring *in situ* phospho histone H3 (ser10). The kit has the following features:

- Quick and efficient procedure, which can be finished within 3 hours.
- Innovative colorimetric assay without the need for radioactivity, electrophoresis, or chromatography.
- Measurement of *in situ* histone H3 phosphorylation at ser10 with no need to prepare cell lysates.
- Microplate format makes the assay suitable for high throughput analysis of reagents that increase or inhibit histone H3 phosphorylation at ser10.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

EpiQuik™ In Situ Histone H3 Phosphorylation (Ser10) Assay Kit is a whole cell-based method for the detection of histone H3 phosphorylation (ser10). In this assay, adherent cells are cultured in

conventional 96-well microplates. After your experimental treatment, cells are fixed and permeabilized. The histone H3 phosphorylation (ser10) is then detected by an anti-phospho histone H3 (ser 10) antibody. The ratio or amount of phospho histone H3 (ser10) can be quantified through HRP conjugated secondary antibody-color development system and is proportional to the intensity of color development.



Schematic Procedure for Using the *EpiQuik™ In Situ* Histone H3 Phosphorylation (Ser10) Assay Kit

PROTOCOL

Before starting, perform the following:

a) Prepare the following required solution (not included): 37% Formaldehyde.
b) Ensure that all buffer solutions are clear in appearance. Shake or vortex if these buffers have precipitates.

1. Inoculate and grow adherent cells in the 96-well microplate to 50-70% confluency. Leave 2-4 wells with no cell inoculation as the blank. Treat cells with the appropriate amount of reagents that may increase or reduce H3 (ser10) phosphorylation for the appropriate time, based on your experiment design.
2. Prepare fixing solution by adding 2.16 ml of 37% formaldehyde to 18 ml of PBS. Remove culture media from the wells with a quick and firm wrist-flick.
3. Immediately add 150 µl of fixing solution slowly to the wells and incubate at room temperature for 15 min. Remove fixing solution from wells with a wrist-flick. While still inverted, tap the plate gently onto absorbent paper to remove any excess fixing reagents still within the wells.
4. Dilute PA1 with distilled water (pH 7.2-7.5) at a 1:10 ratio (e.g., 1 ml of PA1 + 9 ml of distilled water). Wash the wells once (for 2 minutes) with 150 µl of the Diluted PA1.

5. Remove the wash buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution. Add 150 µl of **PA2** to each well and incubate at room temperature for 5 minutes. Meanwhile, prepare the **1% H_2O_2 Solution** by adding 330 µl of **30% H_2O_2 Solution** into 10 ml of **PA2**.
6. Remove **PA2** from wells with a wrist flick. Add 100 µl of the **1% H_2O_2 Solution** into each well and incubate at room temperature for 10 minutes to remove endogenous peroxidase.
7. Remove the **1% H_2O_2 Solution** from the wells with a wrist flick and wash the wells twice with 150 µl of **Diluted PA1**.
8. Remove the wash buffer with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution. Add 150 µl of **PA3** to the wells and incubate at 37°C for 45 minutes. Meanwhile, add 50 µl of **Diluted PA1** to the desired number of control strip wells, followed by adding 1 µl of **Phospho H3^{Ser10} Control** protein at different amounts (ex: 0.5-20 ng, diluted with distilled water) and incubate at room temperature for 30-45 minutes. For the blank wells, do not add any Phospho H3^{Ser10} Control protein.
9. Remove **PA3** with a wrist flick; while still inverted, tap the plate onto absorbent paper. Wash the wells twice with 150 µl of the **Diluted PA1**. For each wash, remove the **Diluted PA1** with a wrist flick; while still inverted, tap the plate onto absorbent paper. Meanwhile, aspirate the solution from control strip wells and wash the wells with 150 µl of **Diluted PA1** three times.
10. Dilute **PA5** (at a 1:1000 ratio) to 1 µg/ml with **PA4**. Add 50 µl of the **Diluted PA5** to the sample wells and **control strip wells**. Incubate at room temperature for 60 min on an orbital shaker (100 rpm).
11. Remove solution from the wells with a wrist flick and wash the wells four times with 150 µl of **1 X PA1**. For each wash, remove **1 X PA1** with a wrist flick; while still inverted, tap the plate onto absorbent paper.
12. Dilute the **PA6** (at a 1:1000 ratio) to 0.2 µg/ml with **1 X PA1**. Add 50 µl of diluted **PA6** to the wells and incubate at room temperature for 30 min.
13. Remove solution from the wells with a wrist flick and wash the wells four times with 150 µl of **1 X PA1**. For each wash, remove **1 X PA1** with a wrist flick, while still inverted, tap the plate onto absorbent paper.
14. Add 100 µl of **PA7** to the wells and incubate at room temperature for 2-10 min away from light. Monitor color development in the sample and control wells until you see a medium blue color.
15. Add 50 µl of **PA8** to the wells and read absorbance on microplate reader at 450 nm.
16. Calculate % H3 phosphorylation (ser 10):

$$\text{Phosphorylation \%} = \frac{\text{OD (treated sample – blank)}}{\text{OD (untreated control – blank)}} \times 100\%$$

17. For an accurate calculation, plot OD value versus amount of **Phospho H3^{ser10} control** and determine the slope as delta OD/ng. Calculate Phospho H3^{ser10} amount using the following formula:

$$\text{Phospho H3}^{ser10}\text{ (ng)} = \frac{\text{OD (sample – blank)}}{\text{slope}}$$

TROUBLESHOOTING

No Signal for Both the Positive Control and the Samples

Reagents are added incorrectly.	Check if reagents are added in order and if any steps of the procedure may have been omitted by mistake.
Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.

No Signal for Only the Sample

Cells are not fixed and permeabilized sufficiently.	Ensure fixation solution and permeabilizing solution are sufficiently added into cells and incubation time is enough.
The protein amount is added into well insufficiently.	Ensure extract contains sufficient amount of protein.

High Background Present for the Blank

The well is not washed enough.	Check if wash at each step is performed according to the protocol.
Overdevelopment.	Decrease development time in step 14.

RELATED PRODUCTS

P-7002 EpiQuik™ Global Histone H3 Phosphorylation (Ser10) Assay Kit (Colorimetric)