



Version 1.0808

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

Catalog No. P-7001

User Guide

***Always use the most updated User
Guide included in your current order.**

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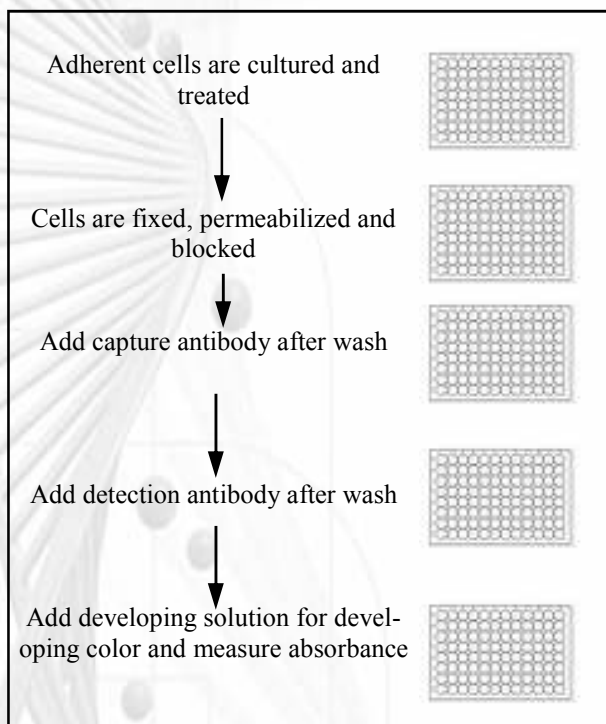
INTRODUCTION

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 AND 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

PRODUCT USE INFORMATION

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.

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

Epigentek guarantees the performance of all products in the manner described in our product instructions.

Epigentek reserves the right to change or modify any product at any time to enhance its performance and design.

EpiQuik™ is a trademark of Epigentek Group Inc.

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)	10 ml
PA5 (capture antibody, 1000 µg/ml)*	9 µl
PA6 (detection antibody, 200 µg/ml)*	10 µl
PA7 (developing solution)	12 ml
PA8 (stop solution)	6 ml
30% H ₂ O ₂ solution	0.5 ml
Phospho H3 ^{ser10} control (20 µg/ml)	15 µl
8-well control strips	2
Microplates	1
User guide	1

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

SHIPPING AND STORAGE

Upon receipt, store **PA6** and **phospho H3^{ser10} control** at -20°C. Store **all other components** at 4°C away from light. The components of the kit are stable for up to 6 months 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

PROTOCOL

Before starting, perform the following:

1. Prepare the following required solutions (not included): 37% formaldehyde.
2. Ensure that all buffers are in clear solution. Shake or vortex if these buffers precipitate.
 1. Inoculate and grow adherent cells in 96-well microplate to 50-70% confluency. Leave 2-4 wells with no cell inoculation as the blank. Treat cells with appropriate amount of agents that may increase or reduce H3 (ser10) phosphorylation for appropriate time.
 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 wrist-flick.
 3. Immediately add 150 μ l 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 agent still within the wells.
 4. Dilute **PA1** with distilled water (pH 7.2-7.5) to the **1 X PA1** and wash wells once (2 min) with 150 μ l of **1 X PA1**.
 5. Remove wash buffer with a wrist flick, while still inverted, tap the plate onto absorbent paper to remove any excess solution. Add 150 μ l of **PA2** to each well and incubate at room temperature for 5 min. Meanwhile, prepare 1% H₂O₂ by adding 330 μ l of 30% H₂O₂ into 10 ml of **PA2**.
 6. Remove **PA2** from wells with a wrist flick. Add 100 μ l of the 1% H₂O₂ solution into each well and incubate at room temperature for 10 min to remove endogenous peroxidase.
 7. Remove the H₂O₂ solution from wells with a wrist flick and wash the wells twice with 150 μ l of **1 X PA1**.
 8. Remove wash buffer with a wrist flick while still inverted, and tap the plate onto absorbent paper to remove any excess solution. Add 150 μ l of **PA3** to the wells and incubate at 37°C for 45 min. Meanwhile, add 50 μ l of **1 X PA1** to the desired number of control strip wells, followed by adding 1 μ l of Phospho H3^{ser10} control protein at the different amount (ex: 0.5-20 ng, diluted with distilled water) and incubate at room temperature for 30-45 min. For blank wells, do not add Phospho H3^{ser10} control protein.

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9. Remove **PA3** with a wrist flick, while still inverted, tap the plate onto absorbent paper. Wash the wells twice 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. Meanwhile, aspirate the solution from control strip wells and wash the wells with 150 μl of 1 X **PA1** for three times.
10. Dilute **PA5** (at 1:1000 ratio) to 1 $\mu\text{g}/\text{ml}$ with **PA4**. Add 50 μl of 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 1:1000 ratio) to 0.2 $\mu\text{g}/\text{ml}$ 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 (blue).
15. Add 50 μl of **PA8** to the wells and read absorbance on microplate reader at 450 nm.
16. Calculate % H3 phosphorylation (ser 10):

$$\text{phosphorylation \%} = \frac{\text{O.D. (treated sample - blank)}}{\text{O.D. (untreated control - blank)}} \times 100\%$$

17. For 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:

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

TROUBLESHOOTING

No Signal for Both the Positive Control and the Samples

Reagents are added incorrectly. Check if reagents are added in order and if 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 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 proteins.

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.

ORDERING INFORMATION

Products	Size	Cat. No.
<i>EpiQuik™ In Situ</i> Histone H3 Phosphorylation (Ser10) Assay Kit	96 assays	P-7001-96

Available Related Products	Cat. No.
<i>EpiQuik™ Global</i> Histone H3 Phosphorylation (Ser10) Assay Kit (Colorimetric)	P-7002

Need more components? You can also order parts separately by calling 1-877-374-4368 or e-mailing sales@epigentek.com.



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