EpiQuik™ Global Histone H3 Phosphorylation (Ser10) Assay Kit (Fluorometric)

Base Catalog # P-7003

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik™* Global Histone H3 Phosphorylation (Ser10) Assay Kit (Fluorometric) is suitable for specifically measuring global histone H3 phosphorylation at ser10 using a variety of mammalian cells (human, mouse, etc.) including fresh and frozen tissues, and cultured adherent and suspension cells.

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KIT CONTENTS

Components	48 assays P-7003-48	96 assays P-7003-96
F1 (10X wash buffer)	10 ml	20 ml
F2 (antibody buffer)	6 ml	12 ml
F3 (detection antibody, 1 mg/ml)*	5 µl	10 µl
F4 (fluoro developer)*	12 µl	24 µl
F5 (fluoro enhancer)*	12 µl	24 µl
F6 (fluoro dilution)	4 ml	8 ml
Standard control (100 µg/ml)*	10 µl	20 µl
8 well sample strips (with frame)	4	9
8 well standard control strips	2	3

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

SHIPPING & STORAGE

Upon receipt, store **F3**, **F4** and **standard control** at –20°C away from light. Store **all other components** at 4°C away from light. The components of the kit should be stable for 6 months when stored properly.

*Note: Check if buffers **F1** and **F2** 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

- ☐ Orbital shaker
- ☐ Pipettes and pipette tips
- ☐ Reagent reservoir
- ☐ Fluorescence microplate reader
- ☐ 15 ml conical tube
- ☐ 1.5 ml microcentrifuge tubes

GENERAL PRODUCT INFORMATION

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

Safety: Suitable lab coat, disposable gloves, and eye protection are required when working with the kit.

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: The *EpiQuik*™ Histone H3 Phosphorylation (Ser10) Assay Kit and methods of use contain proprietary technologies by Epigentek. *EpiQuik*™ is a trademark of EpigenTek Group Inc.

A BRIEF OVERVIEW

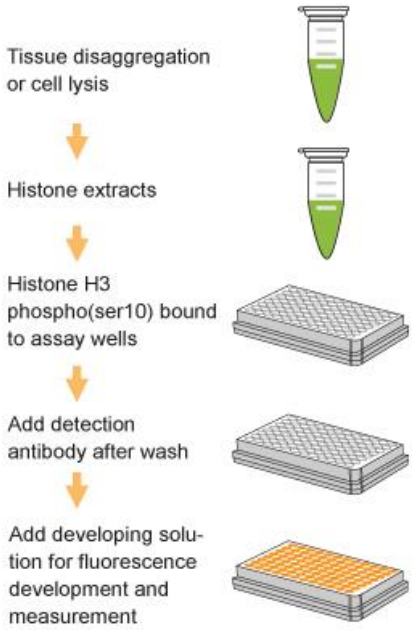
Phosphorylation of histone H3 at serine 10 is conserved through eukaryotes, and an increase in phosphorylation has been shown to correlate with gene activation and cell growth. *In vitro* studies have shown that phosphorylation of histone H3 at ser10 is coupled to acetylation at the nearby Lysine-14 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, while 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 a change in intracellular histone H3 phosphorylation at ser10. Detection of changes in histone H3 phosphorylation at ser10 associated with cell cycle, apoptosis, and inhibitor or activator treatment, would provide useful information for better understanding of the pathological processes of some diseases and for protein kinase-targeted drug development. The *EpiQuik*™ Global Histone H3 Phosphorylation (Ser10) Assay Kit (Fluorometric) provides a tool for measuring global phospho histone H3 (ser10). The 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 (ser10) with the detection limit as low as 2 ng/well.
- A control is conveniently included for the quantification of phosphorylated histone H3 (ser10).
- Strip microplate format makes the assay flexible: manual or high throughput.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*™ Global Histone H3 Phosphorylation (Ser10) Assay Kit (Fluorometric) 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 fluoro development reagent. The ratio of phospho

histone H3 (ser10) is proportional to the relative fluorescent units (RFU). The absolute amount of phospho histone H3 (ser10) can be quantitated by comparing to the standard control.



Schematic Procedure for Using the Global Histone H3 Phosphorylation (Ser10) Assay Kit (Fluorometric)

ASSAY PROTOCOL

1. **a)** Prepare histone extracts from cells/tissues treated or untreated by using your own successful method (acid extraction or high salt extraction).
   b) For your convenience and the best results, EpigenTek offers the *EpiQuik*™ Total Histone Extraction Kit (Cat. # OP-0006) optimized for use in the *EpiQuik*™ modified histone quantification series.
   c) Preparation of histone extracts can also be performed using the attached procedure (See Appendix). Histone extracts can be used immediately or stored at –80°C for future use.

2. 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). Dilute **F1** with distilled water (pH 7.2-7.5) at 1:9 ratio (1 ml of **F1** + 9 ml of distilled water).

3. Add 50 µl of **F2** 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 **F2** to 1 – 100 ng/µl for 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. 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 F1** 3 times.

5. Dilute **F3** (at a 1:1000 ratio) to 1 µg/ml with **F2**. Add 50 µl of diluted **F3** to each well and incubate at room temperature for 60 min on an orbital shaker (100 rpm).

6. Aspirate and wash the wells with 150 µl of **diluted F1** 6 times.

7. Prepare **fluoro-development solution** by adding 1 µl of **F4** and 1 µl of **F5** into each 400 µl of **F6**. Add 50 µl of **fluoro-development solution** into the wells and incubate at room temperature for 1-5 min 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.

8. Calculate % H3 phospho (ser10):

$$\text{Phospho (ser10) \%} = \frac{\text{RFU (treated (tested) sample – blank)}}{\text{RFU (untreated (control) sample – blank)}} \times 100\%$$

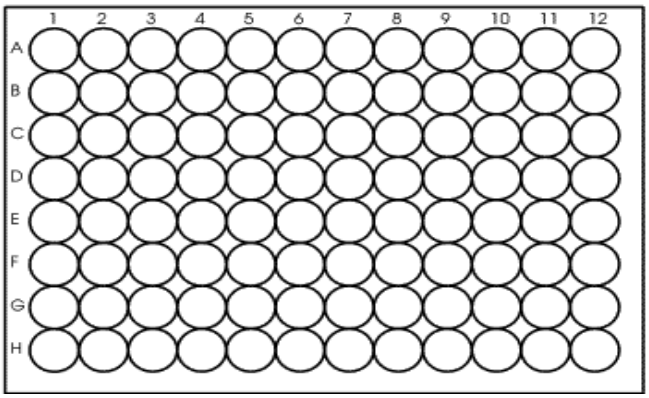
   For the amount quantification, plot RFU versus amount of **standard control** and determine the slope as delta RFU/ng.

   Calculate the amount of phospho (ser10) using the following formula:

$$\text{Amount (ng/mg protein)} = \frac{\text{RFU (sample – blank)}}{\text{Protein (µg)* x slope}} \times 1000$$

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

PLATE CONFIGURATION



- **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.
- Each sample or standard point can be assayed in duplicates or triplicates

Appendix

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 (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 min 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 min at 4°C. Remove supernatant.

   For cells (treated and untreated), harvest cells and pellet the cells by centrifugation at 1000 rpm for 5 min at 4°C. 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°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 min at 4°C. Remove supernatant.

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 min.

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

4. Add 8 volumes (approx. 0.6 ml/10^7 cells or 200 mg tissues) of acetone and leave at –20°C 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 tissues).

6. Quantify the protein concentration. Aliquot the extract and store the extract at –20°C or –80°C.

TROUBLESHOOTING

No Signal for Both the Standard Control and the Samples

Reagents are added incorrectly.	Check if reagents are added in the proper 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 or Very Weak Signal for Only the Standard Control

The amount of standard control is not added into "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 extracted properly.	Ensure the procedure and reagents are correct for the nuclear protein extraction.
The protein amount is added into well insufficiently.	Ensure extract contains 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 from using control protein contaminated tips.
Overdevelopment.	Decrease development time in Step 7.

RELATED PRODUCTS

P-7001	EpiQuik™ In Situ Histone H3 Phosphorylation (Ser10) Assay Kit
P-7002	EpiQuik™ Global Histone H3 Phosphorylation (Ser10) Assay Kit (Colorimetric)
P-7004	EpiQuik™ Global Histone H3 Phosphorylation (Ser28) Assay Kit (Colorimetric)
P-7005	EpiQuik™ Global Histone H3 (Ser28) Assay Kit (Fluorometric)