EpiQuik™ Global Histone H3 Phosphorylation (Ser28) Assay Kit (Fluorometric)

Base Catalog # P-7005

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The EpiQuik™ Global Histone H3 Phosphorylation (Ser28) Assay Kit (Fluorometric) is suitable for specifically measuring global histone H3 phophorylation at ser28 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-7005-48	96 assays P-7005-96
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 are stable for up to 6 months from the date of shipment, 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 (Ser28) Assay Kit (Fluorometric) 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.

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

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 (Ser28) Assay Kit and methods of use contain proprietary technologies by Epigentek. *EpiQuik™* is a trademark of EpigenTek Group Inc.

A BRIEF OVERVIEW

The phosphorylation of histone H3 at serine 28 is conserved in 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 in 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 certain diseases and for protein kinase-targeted drug development. The *EpiQuik™* Global Histone H3 Phosphorylation (Ser28) Assay Kit (Fluorometric) provides a tool for measuring global phospho histone H3 (ser28). 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 (ser28) with a detection limit as low as 0.5 ng/well.
- Conveniently includes a control 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.

PRINCIPLE & PROCEDURE

The *EpiQuik™* Global Histone H3 Phosphorylation (Ser28) 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 color 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 it to the standard control.

Schematic Procedure for Using the Global Histone H3 Phosphorylation (Ser28) Assay Kit (Fluorometric)

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 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 the standard curve, dilute the **standard control** with **F2** 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. 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.w

4. Aspirate and wash the wells three times with 150 µl of **diluted F1** each time.

5. Dilute **F3** (at 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 six times with 150 µl of **diluted F1** each time.

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 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 % histone H3 phospho (ser28):

$$\text{Phospho (ser28) \%} = \frac{\text{RFU (treated (tested) sample – blank)}}{\text{RFU (untreated (control) sample – blank)}} \times 100\%$$

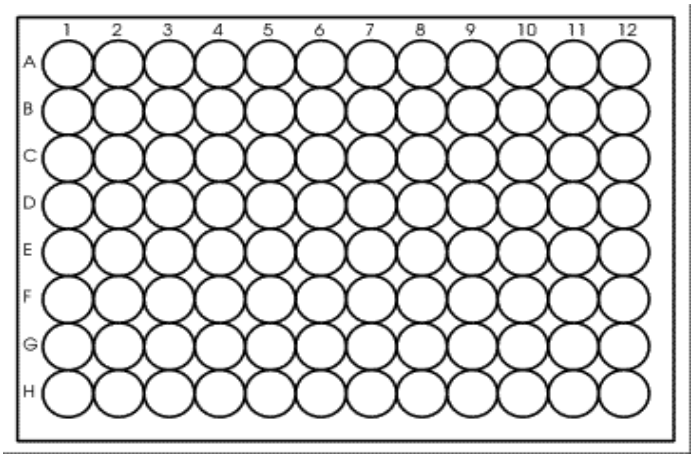
For the amount quantification, plot RFU versus amount of **standard control** and determine the slope as delta RFU/ng.

Calculate the amount of phospho (ser28) 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.

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 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 centrifgation 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 $\mu l/10^7$ 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 $\mu l/10^7$ 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 order and if some steps of the procedure are 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 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 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 by adding the control protein or by 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-7003	EpiQuik™ Global Histone H3 Phosphorylation (Ser10) Assay Kit (Fluorometric)
P-7004	EpiQuik™ Global Histone H3 Phosphorylation (Ser28) Assay Kit (Colorimetric)

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