EpiQuik™ In Situ DNA Damage Assay Kit

Base Catalog # P-6001

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik™ In Situ* DNA Damage Assay Kit is suitable for specifically measuring DNA damage *in situ* through phospho H2AX^{Ser139} detection using cultured adherent cells.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

KIT CONTENTS

Components	96 assays P-6001-096	2 x 96 assays P-6001-192
A1 (10X Wash Buffer)	30 ml	2 x 30 ml
A2 (Permeabilizing Buffer)	30 ml	2 x 30 ml
A3 (Blocking Buffer)	20 ml	2 x 20 ml
A4 (Antibody Buffer)	10 ml	20 ml
A5 (Capture Antibody, 1000 µg/ml)*	10 µl	20 µl
A6 (Detection Antibody, 200 µg/ml)*	20 µl	40 µl
A7 (Developing Solution)	12 ml	24 ml
A8 (Stop Solution)	6 ml	12 ml
30% H_2O_2 Solution	0.5 ml	1 ml
Positive Control (Etoposide, 2 mM)*	10 µl	20 µl
Microplates	1	2

** 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 **A6** and **Positive Control** at –20°C away from light; (2) Store **A1**, **A3**, **A5**, **A7**, and **30% H_2O_2 Solution** at 4°C away from light; (3) Store **all other components** at room temperature. The kit is stable for up to 6 months from the shipment date, when stored properly.

*Note: Check if wash buffer, **A1**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Cell culture medium
- ☐ 37% formaldehyde
- ☐ Variable volume pipette (20-1000 µl) and pipette tips
- ☐ Orbital shaker
- ☐ Microplate reader

GENERAL PRODUCT INFORMATION

Usage Limitation: The *EpiQuik™ In Situ* DNA Damage 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.

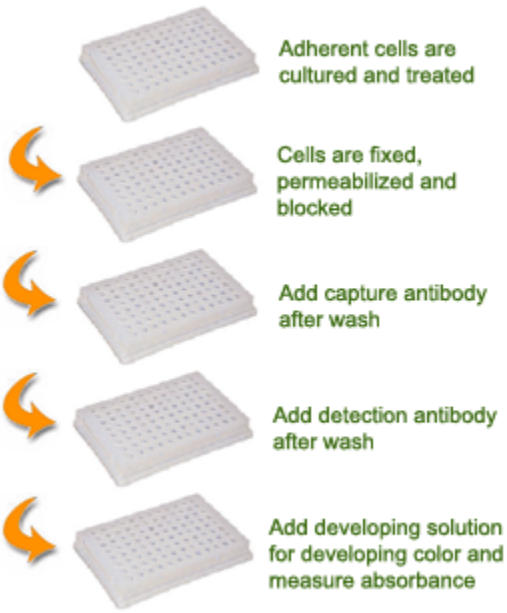
A BRIEF OVERVIEW

DNA double-strand breaks (DSBs) caused by cellular exposure to genotoxic agents or produced by inherent metabolic processes initiate a rapid and highly coordinated series of molecular events resulting in DNA damage signaling and repair. There is increasing evidence for the role of chromatin in DNA damage responses, and several recent studies have implicated histone modifications and chromatin modulation to genome stability. Phosphorylation of histone H2AX to form r-H2AX is one of the earliest chromatin modification events in DNA damage response, and is important for the coordination of signaling and repair activities. Therefore, H2AX phosphorylation at serine 139 can be used as a rapid and sensitive marker for DNA damage and apoptosis. *EpiQuik*™ *In Situ* DNA Damage Assay Kit provides a useful tool for detecting DNA damage or apoptosis by measuring phosphorylation of H2AX (Ser139) *in situ*. 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 H2AX phosphorylation without the need to prepare cell lysates.
- Microplate format makes the assay suitable for high throughput analysis of agents that increase or inhibit DNA damage.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*™ *In Situ* DNA Damage Assay Kit is a whole cell-based assay for the detection of DNA damage and/or apoptosis. In this assay, adherent cells are cultured in conventional 96-well microplates. After your experimental treatment, cells are fixed and permeabilized. The phosphorylation of H2AX at serine 139, the sensitive marker of DNA damage, is then detected by an anti-phospho H2AX^{Ser139} antibody. The ratio or amount of phospho H2AX^{Ser139} 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* DNA Damage Assay Kit

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 the provided 96-well microplate (in the kit) to 50-70% confluency. Leave 2 to 4 wells without cell inoculation as the blank. Treat cells with or without Etoposide (Positive control, 1-10 μM/well) or appropriate amount of agents that may increase or reduce DNA damage for appropriate time (2-24 hours).
2. Prepare the **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 μl of the **Fixing Solution** slowly to the wells and incubate at room temperature for 15 minutes. Remove **Fixing Solution** from the 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 **A1** with distilled water (pH 7.2-7.5) at a 1:10 ratio (e.g., 1 ml of **A1** + 9 ml of distilled water).
5. Wash wells once for 2 minutes with 150 μl of the **Diluted A1**.
6. 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 **A2** 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** into 10 ml of **A2**.
7. Remove **A2** from the 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.

8. Remove the **1% H_2O_2 Solution** from the wells with a wrist flick and wash the wells twice with 150 µl of the **Diluted A1**.
9. 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 **A3** to the wells and incubate at 37°C for 45 minutes.
10. Remove **A3** from the wells with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution. Wash the wells twice with 150 µl of the **Diluted A1**. For each wash, remove the **Diluted A1** from the wells with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution.
11. Dilute **A5** (at a 1:1000 ratio) to 1 µg/ml with **A4**. Add 50 µl of the **Diluted A5** to the wells. Incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).
12. Remove the solution from the wells with a wrist flick and wash the wells four times with 150 µl of the **Diluted A1**. For each wash, remove the **Diluted A1** with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution.
13. Dilute **A6** (at a 1:1000 ratio) to 0.2 µg/ml with **A4**. Add 50 µl of the **Diluted A6** to the wells and incubate at room temperature for 30 minutes.
14. Remove the solution from the wells with a wrist flick and wash the wells four times with 150 µl of the **Diluted A1**. For each wash, remove the **Diluted A1** with a wrist flick; while still inverted, tap the plate onto absorbent paper to remove any excess solution.
15. Add 100 µl of **A7** to the wells and incubate at room temperature for 2-10 minutes away from light. Monitor the color development in the blank, sample, and positive control wells. The color in the positive control wells should change to moderate-brilliant blue, while the color in the blank wells may not change, or may only change slight-blue.
16. Add 50 µl of **A8** to the wells and read absorbance on microplate reader at 450 nm.
17. Calculate the % of DNA damage using the following formula:

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

TROUBLESHOOTING

No Signal for Both the Positive Control and the Samples

Reagents are added incorrectly.	Check if reagents are added in 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 for Only the Sample

Cells are not fixed and permeabilized sufficiently.	Ensure fixation solution and permeabilizing solution are sufficiently added into cells and that incubation time is as specified in protocol.

High Background Present for the Blank

Insufficient washing of the wells.	Check if washing recommendations at each step is performed according to the protocol.
Overdevelopment.	Decrease development time in step 15.

RELATED PRODUCTS

P-3001	EpiQuik™ DNA Methyltransferase Activity/Inhibition Assay Kit
P-3002	EpiQuik™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K4)
P-3003	EpiQuik™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K9)
P-3016	EpiQuik™ In Situ Histone H3-K9 Methylation Assay Kit
P-3017	EpiQuik™ Global Histone H3-K4 Methylation Assay Kit
P-3018	EpiQuik™ Global Histone H3-K9 Methylation Assay Kit
P-3019	EpiQuik™ DNA Demethylase Activity/Inhibition Assay Kit
P-3020	EpiQuik™ Global Histone H3-K27 Methylation Assay Kit