EpiQuik™ In-Situ and Ex-Situ Hydrogen Peroxide (H_2O_2) Assay Kit

Base Catalog # P-6005

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ In-Situ and Ex-Situ Hydrogen Peroxide (H_2O_2) Assay Kit is suitable for accurate measurement of hydrogen peroxide from various samples in-situ and ex-situ in a fast and sensitive manner.

Starting Material: Starting materials can include live cells, extracts from various tissue or cell samples such as cells from a flask or microplate cultured cells, culture supernatant, plasma/serum, and urine samples, etc.

Internal Control: This kit includes an H_2O_2 assay standard, which can be used as a control in quantifying H_2O_2 amounts generated from various samples by comparing the fluorescence intensity of the sample with the standard.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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KIT CONTENTS

Component	96 Assays Cat. #P-6005-096	2 X 96 Assays Cat. #P-6005-192	Storage Upon Receipt
HAB (H_2O_2 Assay Buffer)	14 ml	28 ml	4°C
HAS (H_2O_2 Assay Standard,10 mM)*	10 µl	20 µl	–20°C
HAP (H_2O_2 Assay Probe, 500 X)*	24 µl	48 µl	–20°C
HAE (H_2O_2 Assay Enhancer, 500 X)*	24 µl	48 µl	4°C
8-Well Assay Strips (With Frame)	12	24	4°C
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** Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

The kit is shipped on frozen ice packs at 4°C.

Upon receipt: (1) Store **HAS** and **HAP** at –20°C away from light; (2) Store remaining components (**HAB, HAE**, and **8-Well Assay Strips**) at 4°C away from light.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ 1 X PBS solution
- ☐ Adjustable pipette
- ☐ Aerosol resistant pipette tips
- ☐ Microplate reader capable of reading fluorescence at 530 excitation and 590 emission
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Foil
- ☐ Your sample of interest

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiQuik™ In-Situ and Ex-Situ Hydrogen Peroxide (H_2O_2) Assay Kit is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

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.

Usage Limitation: EpiQuik™ In-Situ and Ex-Situ Hydrogen Peroxide (H_2O_2) Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiQuik™ In-Situ and Ex-Situ Hydrogen Peroxide (H_2O_2) Assay Kit is part of proprietary technologies by EpigenTek Group Inc.

A BRIEF OVERVIEW

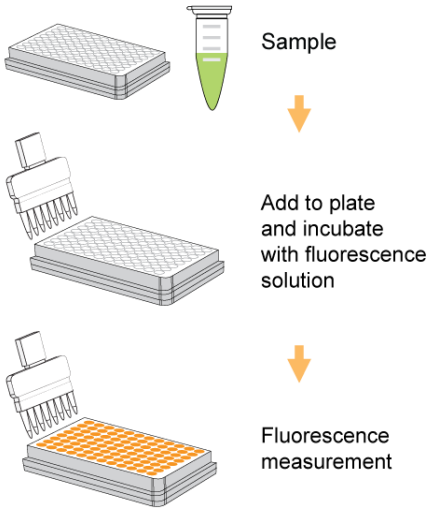
Hydrogen peroxide (H_2O_2) is a reactive oxygen species generated in cellular oxygen metabolism. Abnormal H_2O_2 contributes to oxidative cell damage and the progress of diseases such as inflammation, ischemia, and neurodegeneration. Hydrogen peroxide is a major marker of the ROS release from cell or cell organelles such as mitochondria. H_2O_2 can also be an indicator of the oxidase activity such as NADPH and glucose oxidase as a co-product of oxidase-catalyzed reactions. Thus, it is important to detect H_2O_2 from various biological samples in determining how oxidative stress modulates varied intracellular pathways under physiological and pathological conditions.

The EpiQuik™ In-Situ and Ex-Situ Hydrogen Peroxide (H_2O_2) Assay Kit provides a simple and reproducible method to quantify hydrogen peroxide levels in-situ (living cells) and ex-situ (cell/tissue extracts and body fluids). The kit has the following advantages and features:

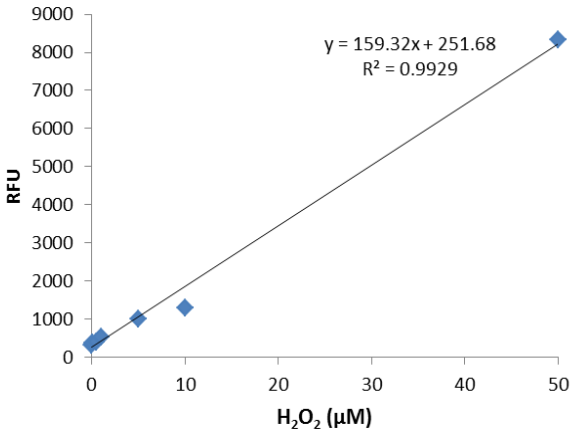
- Fast procedure, which can be finished within 10 min.
- Homogeneous fluorescence assay for both in-situ (live cells) and ex-situ (cell/tissue extract or body fluids) H_2O_2 detection, which is highly sensitive with LOD around 0.03 µM or 1 pg.
- Strip-based microplate format makes the assay flexible via manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The EpiQuik™ In-Situ and Ex-Situ Hydrogen Peroxide (H_2O_2) Assay Kit is designed for the measurement of hydrogen peroxide from various samples in-situ and ex-situ. In the assay with this kit, H_2O_2 assay probe fluorogen 10-Acetyl-3,7-dihydroxy-phenoxazine is incubated with various samples in the strip wells. Hydrogen peroxide contained in or generated from the samples reacts with the H_2O_2 assay probe and produces highly fluorescent oxidation products. The measured intensity of fluorescence from oxidation products is proportional to the amount or concentration of H_2O_2.



Schematic procedure of EpiQuik™ In-Situ and Ex-Situ Hydrogen Peroxide (H_2O_2) Assay Kit



Illustrated standard curve generated with the H_2O_2 assay standard.

PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Pre-preparation of Sample

Cell/tissue extract: For cells, directly resuspend 1 million cells in 0.5 ml ice-cold 1 X PBS. Homogenize cells in a Dounce homogenizer or with a pestle in a 1.5 ml microcentrifuge tube. For tissues, cut the sample into small pieces (1-2 mm^3) with a scalpel or scissors, place approximate 2 mg minced tissue into homogenizer or microcentrifuge tube containing 1 ml PBS for homogenizing. Centrifuge 10,000 rpm for 5 min to remove debris. Cell/tissue extracts can be assayed in undiluted form or with a proper amount determined by series dilution if necessary.

Cell culture supernatant, plasma, serum or urine: centrifuge at 10,000 rpm for 5 min to remove insoluble particles. The supernatant can be assayed directly or in a proper assay amount determined by series dilution if necessary.

Note: *1) The sample should not contain DTT, which may interfere with fluorescence detection; 2) A over-large amount of H_2O_2 (e.g. >50 μM) may affect further fluorescent conversion and lead to a reduction of signal intensity. It thus is necessary to test the proper amount of the sample with several different dilutions.*

Live cells: Incubate cells in 96-well microplate in a monolayer form. A positive control should be set up by treating cells with 100 uM of H_2O_2 in 2-4 wells. After incubation, wash the plate one time with 1X PBS. 2-4 well blank without cells and 2-4 well positive controls containing H_2O_2 treated cells should be set up.

H_2O_2 standard curve Suggested Standard Curve Preparation: First, prepare **Diluted HAS** (H_2O_2 Assay Standard) to 50 µM [ex: use 1 µl of **HAS** + 199 µl of **HAB (**H_2O_2 Assay Buffer)] and 5 µM [ex:

use 1 µl of the 50 µM **HAS** + 9 µl of **HAB**], respectively. Then, further prepare 7 different concentrations of the **Diluted HAS** into 0.05, 0.1, 0.5, 1, 5, 10 and 50 µM according to the following dilution chart:

Tube	HAS (5 µM)	HAS (50 µM)	HAB	Resulting Final HAS Concentration (µM)
1	1.0 µl		99.0 µl	0.05
2	1.0 µl		49.0 µl	0.1
3	1.0 µl		9.0 µl	0.5
4		1.0 µl	49.0 µl	1.0
5		1.0 µl	9.0 µl	5.0
6		1.0 µl	4.0 µl	10.0
7		4.0 µl	0.0 µl	50.0

Note: *The diluted* ***HAS (****H_2O_2 Assay Standard) is unstable and should be used within the same day of the assay.*

For In-situ Assay

1. Prepare the **Fluorescence Solution** by adding 1 µl of **HAP** (H_2O_2 Assay Probe) and 1 µl of **HAE (**H_2O_2 Assay Enhancer) into each 499 µl of **HAB** (H_2O_2 Assay Buffer).

2. After washing the cell cultured plate with PBS, add 100 µl of **Fluorescence Solution** into each well. Cover with foil and incubate at room temperature for 5-10 min away from light. The color in the control wells (cells containing the higher concentrations of H_2O_2) may turn pink during this period.

3. Measure and read fluorescence on a fluorescence microplate reader at $530_{EX}/590_{EM}$ nm.

   Note: *1). 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.*

   2). A relative comparison between the different samples or between treated and untreated samples can be made by comparing the delta RFU intensity of the samples. For example: H_2O_2 *% = RFU [sample A (treated)-blank]/RFU[sample B (control)-blank)] X 100%.*

   3). For quantitative measurement, a standard curve should be generated with use of included strips as described in step 3 of "For Ex-situ Assay section" according to the setup illustrated in the table 1. For calculation, the first, Plot RFU value versus amount of ***HAS*** H_2O_2 *Assay Standard and determine the slope as delta RFU/µM. then calculate the* H_2O_2 *amount according the following formula:* H_2O_2 *(µM) = RFU (sample-blank)/slope.*

For Ex-situ Assay

1. Predetermine the number of strip wells required for your experiment. Carefully remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

2. Prepare the **Fluorescence Development Solution** by adding 1 µl of **HAP** (H_2O_2 Assay Probe) and 1 µl of **HAE (**H_2O_2 Assay Enhancer) into each 499 µl of **HAB** (H_2O_2 Assay Buffer).

3. Add the following components to the corresponding wells according to this chart:

Well Type	Component	Amount/Well
Blank Wells	**HAB**	20 µl
Standard Wells	**HAB**	19 µl
	Each of Diluted **HAS**	1µl
Sample Wells	Sample	20 µl

4. Add 100 µl of the **Fluorescence Development Solution** into the wells. Cover with foil and incubate at room temperature for 5-10 minutes away from light. The color in the standard wells containing the higher concentrations of H_2O_2 may turn pink during this period.

5. 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.*

6. Use the following formula to calculate the concentration of H_2O_2. Plot RFU value versus amount of **HAS** H_2O_2 Assay Standard and determine the slope as delta RFU/µM.

$$H_2O_2\ (\mu M/\ unit) = \frac{Sample\ RFU - Blank\ RFU}{Slope \times Unit\ of\ Sample^*}$$

**A Unit of sample: 1 million cells or 1 mg tissue for cell/tissues or 1 ml for body fluids used in assay. If the sample amount used for the assay is 20 µl from 0.5 ml solution containing 1 million cells or 1 mg tissue, the unit of sample in the formula is 0.04*

SUGGESTED STRIP WELL SETUP

Table 1. The suggested strip-well plate setup for blank, standard curve, and the samples can be measured in duplicate from the columns 3-12.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Sample	Sample								
B	0.05 µM	0.05 µM	.....	.....								
C	0.1 µM	0.1 µM										
D	0.5 µM	0.5 µM										
E	1 µM	1 µM										
F	5 µM	5 µM										
G	10 µM	10 µM										
H	50 µM	50 µM										

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
No Signals for both standard and samples	Reagents are added incorrectly.	Check if reagents are added in the proper order and if any steps in the protocol 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.
	Insufficient input materials.	Ensure that a sufficient amount of sample and standards is added into the wells.
	Incorrect fluorescence reading.	Check if appropriate fluorescence filters ($530_{EX}/590_{EM}$) are used.
	Kit was not stored or handled properly.	Ensure all components of the kit were stored at the appropriate temperatures and the cap is tightly closed after each opening or use.
No signals only for samples	The amount of the sample added is insufficient.	Ensure a sufficient amount of sample is added into the reaction.
High background present in the blank wells	Contaminated by standard or samples	Ensure the well is not contaminated from adding the standard and samples accidentally or from using contaminated tips.

RELATED PRODUCTS

OP-0001	EpiQuik™ Superoxide Dismutase Activity/Inhibition Assay Kit (Colorimetric)
P-6001	EpiQuik™ In Situ DNA Damage Assay Kit
P-6003	EpiQuik™ 8-OHdG DNA Damage Quantification Direct Kit (Colorimetric)
P-6004	EpiQuik™ 8-OHdG DNA Damage Quantification Direct Kit (Fluorometric)
P-6008	EpiQuik™ 8-OhG RNA Damage Quantification Direct Kit (Colorimetric)