SensoLyte® ADHP Hydrogen Peroxide Assay Kit
Fluorimetric

Revision Number:1.1	Last Revised: October 2014
Catalog #	**AS-71112**
Kit Size	500 Assays (96-well) or 1250 Assays (384-well)

- ***Convenient Format:*** Complete kit includes all the assay components.
- ***Optimized Performance:*** Optimal conditions for quantifying hydrogen peroxide and detecting oxidase.
- ***Enhanced Value:*** Less expensive than the sum of individual components.
- ***High Speed:*** Minimal hands-on time.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	ADHP	10 mM, 250 µL
Component B	H_2O_2 standard	1 vial
Component C	Assay buffer	60 mL
Component D	HRP, Horseradish peroxidase	5 vials, 100µL/vial

Other Materials Required (but not provided)

- 96-well or 384-well microplate: Black, flat-bottom microplates with non-binding surface.
- Fluorescence microplate reader: Capable of detecting emission at 590 nm with excitation at 530-560 nm.

Storage and Handling

- For convenience, Component C can be stored at room temperature.

Introduction

Reactive oxygen species (ROS) play an important role in a variety of biological events, such as inflammation, ischemia and reperfusion, and neurodegeneration. Hydrogen peroxide (H_2O_2) is membrane permeable and is more stable than other ROS. It is often chosen to represent the ROS released by cell or cell organelles (e.g. mitochondria,[1] activated leukocytes[2]). H_2O_2 is also a co-product of many oxidase-catalyzed reactions. Consequently, it can serve as an indicator of the activity of oxidases (e.g. NADPH oxidase[3], glucose oxidase[4], and monoamine oxidase[5]).

The SensoLyte® ADHP Hydrogen Peroxide Assay Kit provides a convenient, highly sensitive fluorescent assay for quantifying H_2O_2 in solutions, in cell extracts and in live cells. In the enzyme-coupled reaction, non-fluorescent ADHP (10-Acetyl-3, 7-dihydroxyphenoxazine) can be oxidized to the strongly fluorescent resorufin in presence of H_2O_2 and horseradish peroxidase (HRP). The signal of resorufin can be easily read by a fluorescence microplate reader at Ex/Em=530-560 nm/590 nm.

Protocol

Note: Warm all kit components to room temperature before starting the experiment.

1. Prepare stock solution.

- H_2O_2 stock solution (1 M): Add 100 µL of deionized water into the H_2O_2 vial (Component B) to get 1 M stock solution. Store this stock solution tightly capped at 4°C.

2. Set up the H_2O_2 standard curve (Optional).

- Dilute 1 M H_2O_2 stock solution to 40 µM in assay buffer (Component C). Perform 2-fold serial dilutions with the assay buffer to get 20, 10, 5, 2.5, 1.25, and 0.63 µM H_2O_2 solutions. Add 50 µL/well of the serially diluted H_2O_2 solution to a 96-well plate or 20 µL/well to a 384-well plate. Include a negative control that does not contain any H_2O_2.

3. Prepare test samples.

- Add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of samples (e.g. mitochondria[1], activated leukocytes[2], monoamine oxidase with its substrate benzylamine[3]).

Note: Extremely large amount of H_2O_2 (e.g. >100 µM) may further convert fluorescent resorufin to non-fluorescent resazurin and lead to reduction of fluorescence signal. It is necessary to test your sample with several different dilutions.

4. Prepare ADHP reaction mixture.

- Prepare fresh ADHP reaction mixture according to the following Table 1 and keep away from light.

Table 1. ADHP reaction mixture for one 96-well plate (100 assays)

Components	Volume
ADHP (Component A)	50 µL
HRP (Component D)	100 µL
Assay buffer (Component C)	4.85 mL
Total volume	5 mL

Note 1: This reaction mixture can detect 0.1 nmol of H_2O_2 with a linear range of up to 2 nmol (Figure 1). Lowering the ADHP concentration in the reaction mixture can decrease background and increase assay sensitivity. 10 μM ADHP can detect as low as 2 pmol of H_2O_2[2]. 2 μM ADHP was used to detect H_2O_2 produced by mitochondria[1].
Note 2: You may change the assay buffer to any buffer appropriate for your samples. For example, you may use Krebs-Ringer phosphate for detecting H_2O_2 released from activated human leukocytes[2] or modified buffer for mitochondria[1]. You may also add stimulating reagents in the reaction mixture.[2]

5. Detect H_2O_2.

5.1 Add 50 μL/well (96-well plate) or 20 μL/well (384-well plate) of ADHP reaction mixture. Mix the reagents by gently shaking the plate for 30 sec.

5.2 Incubate the reaction at the desired temperature for 15-30 min. Measure emission at 590 nm with excitation at 530-560 nm.

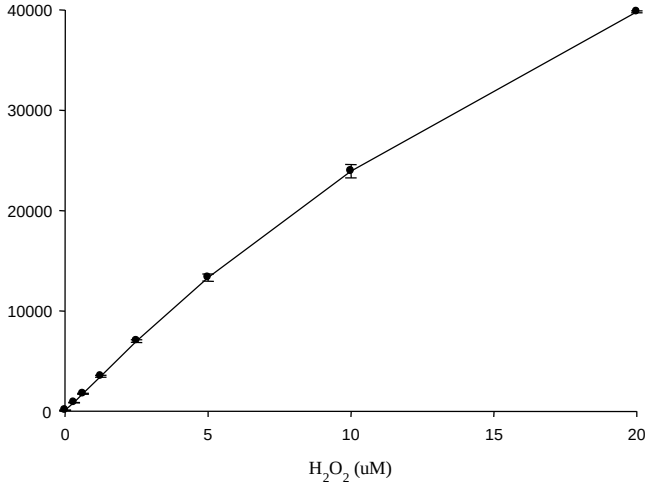

Figure 1. The standard curve of H_2O_2 H_2O_2 was serially diluted and detected according to the above protocol. With the total assay volume of 100 μL, the assay can detect as low as 1 μM (0.1 nmol) H_2O_2 with a linear range up to 20 μM (2 nmol) (R^2>0.98). (n=2, mean±S.D.)

References

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4. Sanchez, F.A. et al. *Anal. Biochem.* **187**, 129 (1990)
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