



# SensoLyte<sup>®</sup> ADHP Hydrogen Peroxide Assay Kit *\*Fluorimetric\**

Revision Number:1.1	Last Revised: October 2014
<b>Catalog #</b>	<b>AS-71112</b>
<b>Kit Size</b>	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 $\mu$ L
Component B	H <sub>2</sub> O <sub>2</sub> standard	1 vial
Component C	Assay buffer	60 mL
Component D	HRP, Horseradish peroxidase	5 vials, 100 $\mu$ L/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,<sup>1</sup> activated leukocytes<sup>2</sup>).  $H_2O_2$  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<sup>3</sup>, glucose oxidase<sup>4</sup>, and monoamine oxidase<sup>5</sup>).

The SensoLyte<sup>®</sup> 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  $\mu$ L 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  $\mu$ M 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  $\mu$ M  $H_2O_2$  solutions. Add 50  $\mu$ L/well of the serially diluted  $H_2O_2$  solution to a 96-well plate or 20  $\mu$ L/well to a 384-well plate. Include a negative control that does not contain any  $H_2O_2$ .

### 3. Prepare test samples.

- Add 50  $\mu$ L/well (96-well plate) or 20  $\mu$ L/well (384-well plate) of samples (e.g. mitochondria<sup>1</sup>, activated leukocytes<sup>2</sup>, monoamine oxidase with its substrate benzylamine<sup>3</sup>).

Note: Extremely large amount of  $H_2O_2$  (e.g. >100  $\mu$ M) 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 $\mu$ L
HRP (Component D)	100 $\mu$ L
Assay buffer (Component C)	4.85 mL
Total volume	5 mL

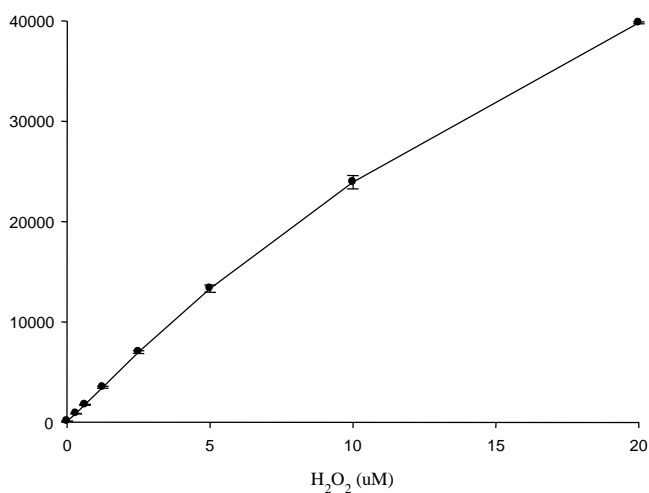
**Note 1:** This reaction mixture can detect 0.1 nmol of H<sub>2</sub>O<sub>2</sub> with a linear range of up to 2 nmol (Figure 1). Lowering the ADHP concentration in the reaction mixture can decrease background and increase assay sensitivity. 10 μM ADHP can detect as low as 2 pmol of H<sub>2</sub>O<sub>2</sub>. 2 μM ADHP was used to detect H<sub>2</sub>O<sub>2</sub> produced by mitochondria<sup>1</sup>.

**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<sub>2</sub>O<sub>2</sub> released from activated human leukocytes<sup>2</sup> or modified buffer for mitochondria<sup>1</sup>. You may also add stimulating reagents in the reaction mixture.<sup>2</sup>

## 5. Detect H<sub>2</sub>O<sub>2</sub>.

**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<sub>2</sub>O<sub>2</sub>. H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> with a linear range up to 20 μM (2 nmol) ( $R^2 > 0.98$ ). (n=2, mean±S.D.)

---

## References

1. Votyakova, T.V. and Reynolds I.J., *J. Neurochem.* **79**, 266 (2001)
2. Mohanty, J.G. et al. *J. Immunol. Methods.* **202**, 133 (1997)
3. Zhou, M. et al. *Anal. Biochem.* **253**, 162 (1997)
4. Sanchez, F.A. et al. *Anal. Biochem.* **187**, 129 (1990)
5. Youdim, M.B. and Tenne M., *Methods. Enzymol.* **142**, 617 (1987)