SensoLyte® ADHP Hydrogen Peroxide Assay Kit

*Fluorimetric*

<table>
<thead>
<tr>
<th>Revision Number: 1.1</th>
<th>Last Revised: October 2014</th>
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<tbody>
<tr>
<td><strong>Catalog #</strong></td>
<td>AS-71112</td>
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<tr>
<td><strong>Kit Size</strong></td>
<td>500 Assays (96-well) or 1250 Assays (384-well)</td>
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- **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

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component A</td>
<td>ADHP</td>
<td>10 mM, 250 μL</td>
</tr>
<tr>
<td>Component B</td>
<td>H₂O₂ standard</td>
<td>1 vial</td>
</tr>
<tr>
<td>Component C</td>
<td>Assay buffer</td>
<td>60 mL</td>
</tr>
<tr>
<td>Component D</td>
<td>HRP, Horseradish peroxidase</td>
<td>5 vials, 100μL/vial</td>
</tr>
</tbody>
</table>

**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$_2$O$_2$) 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, activated leukocytes$^2$). H$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ stock solution (1 M): Add 100 µL of deionized water into the H$_2$O$_2$ vial (Component B) to get 1 M stock solution. Store this stock solution tightly capped at 4°C.

2. Set up the H$_2$O$_2$ standard curve (Optional).
   - Dilute 1 M H$_2$O$_2$ 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$_2$O$_2$ solutions. Add 50 µL/well of the serially diluted H$_2$O$_2$ 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$_2$O$_2$.

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$_2$O$_2$ (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)

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume</th>
</tr>
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<tbody>
<tr>
<td>ADHP (Component A)</td>
<td>50 µL</td>
</tr>
<tr>
<td>HRP (Component D)</td>
<td>100 µL</td>
</tr>
<tr>
<td>Assay buffer (Component C)</td>
<td>4.85 mL</td>
</tr>
<tr>
<td>Total volume</td>
<td>5 mL</td>
</tr>
</tbody>
</table>
Note 1: This reaction mixture can detect 0.1 nmol of H$_2$O$_2$ 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$_2$O$_2$. 2 µM ADHP was used to detect H$_2$O$_2$ produced by mitochondria.

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$_2$O$_2$ released from activated human leukocytes or modified buffer for mitochondria. You may also add stimulating reagents in the reaction mixture.

5. Detect H$_2$O$_2$.

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$_2$O$_2$. H$_2$O$_2$ 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$_2$O$_2$ with a linear range up to 20 µM (2 nmol) ($R^2>$0.98). (n=2, mean±S.D.)

References