Peroxidase Assay Kit

Catalog Number KA1620
100 assays
Version: 02

Intended for research use only
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Introduction

Intended Use

Features:
- Use as little as 10 µL samples. Linear detection range: colorimetric assays 4 to 1000 IU/L, fluorimetric assays 0.8 to 25 IU/L peroxidase.

Principle of the Assay

PEROXIDASES (EC number 1.11.1.x) catalyze the following oxidation-reduction reactions:

ROOR' + electron donor (2 e^-) + 2H^+ → ROH + R'OH

For many peroxidases the optimal substrate is hydrogen peroxide (H₂O₂), but others are more active with organic hydroperoxides such as lipid peroxides. In the cell, peroxidases destroy toxic hydroxide radicals that are formed as byproducts during aerobic respiration. The peroxidases represent a large family of enzymes that are found in animals (e.g. myeloperoxidase-like enzymes), plant, fungi and bacteria (cytochrome-c peroxidase like enzymes such as horseradish peroxidase). Simple, direct and automation-ready procedures for determining peroxidase activity find wide applications. Peroxidase Assay Kit uses H₂O₂ and an electron donor dye that forms a pink color during the peroxidase reaction. The optical density (570nm) or fluorescence intensity (λexc = 530nm, λem = 590nm) is a direct measure of the enzyme activity.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer (pH 7.0)</td>
<td>20 mL</td>
</tr>
<tr>
<td>Stabilized H₂O₂</td>
<td>100 µL 3%</td>
</tr>
<tr>
<td>Calibrator</td>
<td>5 mL (equivalent to 1000 IU/L)</td>
</tr>
<tr>
<td>Dye Reagent</td>
<td>60 µL</td>
</tr>
<tr>
<td>Stop Reagent</td>
<td>12 mL</td>
</tr>
</tbody>
</table>

Storage Instruction

Store Assay Buffer and Dye Reagent at -20°C, all other reagents at 4°C.
Shelf life: 3 months after receipt.

Materials Required but Not Supplied

Pipeting devices, centrifuge tubes, clear flat bottom 96/384-well plates for colorimetric assays, black 96/384-well plate for fluorimetric assays and plate reader

Precautions for Use

- Precautions
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.
- This assay is based on a kinetic reaction, the use of a multi-channel pipettor for adding the Working Reagent and Stop Reagent is recommended.
Assay Protocol

Reagent Preparation

Bring all reagents to room temperature prior to assay. Dilute 3% \( \text{H}_2\text{O}_2 \) in Assay Buffer to 0.6% and use within one hour.

Sample Preparation

Samples can be prepared according to established methods [1-3]. It is prudent to test multiple sample dilutions to ensure activity is in the linear range.

Assay Procedure

- **96-Well Colorimetric Assay Procedure**
  Use clear flat-bottom plates for colorimetric assays
  1. Transfer 200 µL \( \text{H}_2\text{O} \) and 200 µL Calibrator into two wells of a clear flat-bottom 96-well plate. Transfer 10 µL \( \text{H}_2\text{O} \) (sample blank), 10 µL sample to separate wells. Prepare fresh Working Reagent for each reaction well by mixing 95 µL Assay Buffer, 0.5 µL Dye Reagent and 0.5 µL freshly diluted 0.6% \( \text{H}_2\text{O}_2 \). Add 90 µL Working Reagent to each sample well. Tap plate to mix and incubate for 10 min at room temperature.
  2. Add 100 µL Stop Reagent to the sample blank and sample wells. Tap plate to mix and read OD570nm. **Note:** if Sample OD values are higher than that of the Calibrator, dilute sample in Assay Buffer, repeat assay and multiply results by the dilution factor. Peroxidase activity is calculated from the OD values of the sample, sample blank, calibrator and \( \text{H}_2\text{O} \) wells.

- **96-Well Fluorimetric Assay Procedure**
  Use black flat-bottom plates. If desirable, a peroxidase standard (e.g. Sigma Aldrich Cat# P6140 horseradish peroxidase) can be run together with the samples.
  1. Transfer 10 µL \( \text{H}_2\text{O} \), 10 µL sample to separate wells. Prepare fresh Working Reagent for each sample well by mixing 95 µL Assay Buffer, 0.5 µL Dye Reagent and 0.5 µL freshly diluted 0.6% \( \text{H}_2\text{O}_2 \). Add 90 µL Working Reagent to each well. Tap plate to mix and incubate for 10 min at room temperature.
  2. Add 100 µL Stop Reagent to the sample blank and sample wells. Tap plate to mix and read fluorescence at 590nm (λexc= 530nm).

- **384-Well Fluorimetric Assay Procedure**
  Use 5 µL \( \text{H}_2\text{O} \), 5 µL sample, 35 µL Working Reagent prepared from 38 µL Assay Buffer, 0.2 µL Dye Reagent, 0.2 µL 0.6% \( \text{H}_2\text{O}_2 \). Use 40 µL Stop Reagent.
Data Analysis

Calculation of Results

\[
\text{Peroxidase Activity} = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{OD}_{\text{CALIBRATOR}} - \text{OD}_{\text{H2O}}} \times 1000 \text{ (IU/L)}
\]

Unit definition: one unit of enzyme will catalyze the oxidization by \( \text{H}_2\text{O}_2 \) of 1 \( \mu \)mole dye reagent per min under the assay conditions.

96-well Colorimetric Assay

384-well Fluorimetric Assay
References