# Peroxidase Assay Kit (Colorimetric/Fluorometric)

LS-K188-100 (100 Tests) • **Storage at -20°C**

LSBio
LifeSpan BioSciences, Inc.

## Introduction

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

$$ROOR' + \text{electron donor } (2\ e^-) + 2H^+ \rightarrow ROH + R'OH$$

For many peroxidases the optimal substrate is hydrogen peroxide ($H_2O_2$), 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 a horseradish peroxidase). Simple, direct and automation-ready procedures for determining peroxidase activity find wide applications. This peroxidase assay uses $H_2O_2$ and an electron donor dye that forms a pink color during the peroxidase reaction. The optical density (570nm) or fluorescence intensity ($\lambda_{ex/em}$ = 530/590nm) is a direct measure of the enzyme activity.

## Key Features

- Fast and sensitive. Use as little as 10 µL samples. Linear detection range: colorimetric assays 2 to 50 U/L, fluorometric assays 0.1 to 5 U/L peroxidase.
- Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

## Applications

- Peroxidase activity determination in biological samples (e.g. plasma, serum, urine, tissue and culture media.)

## Components

| Component | K188-100 |
|---|---|
| | 100 Tests |
| Assay Buffer | 20 mL |
| Stop Reagent | 12 mL |
| Resorufin | 1.5 mL |
| Dye Reagent | 60 µL |
| 3% Stabilized $H_2O_2$ | 100 µL |

## Materials Not Supplied

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), black 96-well plates (e.g. Greiner Bio-One, cat#655900) and plate reader capable of either measuring absorbance between 560-590 nm or fluorescence at $\lambda_{ex/em}$= 530/585 nm.

## Storage

The kit is shipped at room temperature. Store the Assay Buffer, Dye Reagent and Resorufin at -20 °C. The 3% Stabilized $H_2O_2$ should be stored between -20°C and 4°C. The Stop Reagent can be stored at any temperature between -20°C and room temperature. Shelf life: 12 month after receipt.

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## Assay Procedure

### 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.

### Reagent Preparation

Bring all reagents to room temperature prior to assay. Dilute 3% $H_2O_2$ in Assay Buffer to 0.6% and use within one hour.

### Colorimetric Procedure

1. Transfer 100 µL $H_2O$ and 100 µL Resorufin into two wells of a clear flat-bottom 96-well plate.

   Transfer 10 µL $H_2O$ (sample blank), 10 µL sample to separate wells. Prepare enough Working Reagent (WR) for all sample wells by mixing, for each well, 95 µL Assay Buffer, 0.5 µL Dye Reagent and 0.5 µL freshly diluted 0.6% $H_2O_2$. 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 all wells. Tap plate to mix and read $OD_{570nm}$.

### Fluorometric Procedure

For fluorometric assays, the linear detection range is 0.1 to 5 U/L. Dilute the Resorufin 1:10 in $dH_2O$.

1. Transfer 100 µL $H_2O$ and 100 µL diluted Resorufin into two wells of a black flat-bottom 96-well plate.

   Transfer 10 µL $H_2O$, 10 µL sample to separate wells. Prepare enough Working Reagent (WR) for all sample wells by mixing, for each well, 95 µL Assay Buffer, 0.5 µL Dye Reagent and 0.5 µL freshly diluted 0.6% $H_2O_2$. 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 all wells. Tap plate to mix and read fluorescence ($\lambda_{ex/em}$= 530/585 nm).

### Calculations

The peroxidase activity in a sample is computed as follows:

$$\text{Peroxidase Activity} = \frac{R_{SAMPLE} - R_{BLANK}}{R_{RESORUFIN} - R_{H2O}} \times \frac{[\text{Resorufin}]\ (\mu M)}{t\ (\text{min})} \times \frac{\text{Reaction Vol}\ (\mu L)}{\text{Sample Vol}\ (\mu L)} \times n$$

$$= \frac{R_{SAMPLE} - R_{BLANK}}{R_{RESORUFIN} - R_{H2O}} \times [\text{Resorufin}]\ (\mu M) \times n \quad (U/L)$$

where $R_{SAMPLE}$, $R_{BLANK}$, $R_{RESORUFIN}$ and $R_{H2O}$ are OD or fluorescence readings of the Sample, Sample Blank, Resorufin and Water respectively. *n* is the sample dilution factor. The [Resorufin] is 50 µM for colorimetric assays and 5 µM for fluorometric assays. The Reaction Vol is 100 µL and the Sample Vol is 10 µL. Notes: if Sample OD or fluorescence values are higher than that of the Resorufin, dilute sample in Assay Buffer, repeat assay and multiply results by the dilution factor, *n*.

Unit definition: one unit of enzyme will catalyze the formation of 1 µmole resorufin per min under the assay conditions.

## General Considerations

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.

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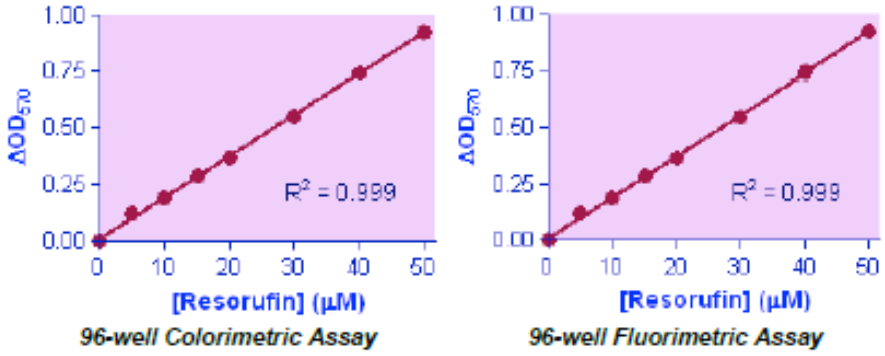
## Sample Data

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