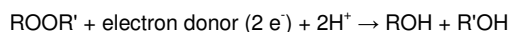


## QuantiChrom™ Peroxidase Assay Kit (Cat# DPOD-100)

### Quantitative Colorimetric/Fluorimetric Peroxidase Determinations

#### DESCRIPTION

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



For many peroxidases the optimal substrate is hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), 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 as horseradish peroxidase).

Simple, direct and automation-ready procedures for determining peroxidase activity find wide applications. BioAssay Systems' peroxidase assay uses  $\text{H}_2\text{O}_2$  and an electron donor dye that forms a pink color during the peroxidase reaction. The optical density (570nm) or fluorescence intensity ( $\lambda_{\text{exc}} = 530\text{nm}$ ,  $\lambda_{\text{em}} = 590\text{nm}$ ) is a direct measure of the enzyme activity.

#### KEY FEATURES

Use as little as 10  $\mu\text{L}$  samples. Linear detection range: colorimetric assays 4 to 1000 IU/L, fluorimetric assays 0.8 to 25 IU/L peroxidase.

#### KIT CONTENTS

**Assay Buffer (pH 7.0):** 20 mL      **Dye Reagent:** 60  $\mu\text{L}$   
**Stabilized  $\text{H}_2\text{O}_2$ :** 100  $\mu\text{L}$  3%      **Stop Reagent:** 12 mL  
**Calibrator:** 5 mL (equivalent to 1000 IU/L)

**Storage conditions.** The kit is shipped on blue ice. Store Assay Buffer and Dye Reagent at  $-20^\circ\text{C}$ , all other reagents at  $4^\circ\text{C}$ . Shelf life of three months after receipt.

**Precautions:** reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

#### PROCEDURES

**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%  $\text{H}_2\text{O}_2$  in Assay Buffer to 0.6% and use within one hour.

**96-Well Colorimetric Assay Procedure.** Use clear flat-bottom plates for colorimetric assays

1. Transfer 200  $\mu\text{L}$   $\text{H}_2\text{O}$  and 200  $\mu\text{L}$  Calibrator into two wells of a clear flat-bottom 96-well plate.

Transfer 10  $\mu\text{L}$   $\text{H}_2\text{O}$  (sample blank), 10  $\mu\text{L}$  sample to separate wells. Prepare fresh Working Reagent for each reaction well by mixing 95  $\mu\text{L}$  Assay Buffer, 0.5  $\mu\text{L}$  Dye Reagent and 0.5  $\mu\text{L}$  freshly diluted 0.6%  $\text{H}_2\text{O}_2$ . Add 90  $\mu\text{L}$  Working Reagent to each sample well. Tap plate to mix and incubate for 10 min at room temperature.

2. Add 100  $\mu\text{L}$  Stop Reagent to the sample blank and sample wells. Tap plate to mix and read  $\text{OD}_{570\text{nm}}$ .

**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.

$$\text{Peroxidase Activity} = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{OD}_{\text{CALIBRATOR}} - \text{OD}_{\text{H}_2\text{O}}} \times 1000 \quad (\text{IU/L})$$

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

**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  $\mu\text{L}$   $\text{H}_2\text{O}$ , 10  $\mu\text{L}$  sample to separate wells. Prepare fresh Working Reagent for each sample well by mixing 95  $\mu\text{L}$  Assay Buffer, 0.5  $\mu\text{L}$  Dye Reagent and 0.5  $\mu\text{L}$  freshly diluted 0.6%  $\text{H}_2\text{O}_2$ . Add 90  $\mu\text{L}$  Working Reagent to each well. Tap plate to mix and incubate for 10 min at room temperature.
2. Add 100  $\mu\text{L}$  Stop Reagent to the sample blank and sample wells. Tap plate to mix and read fluorescence at 590nm ( $\lambda_{\text{exc}} = 530\text{nm}$ ).

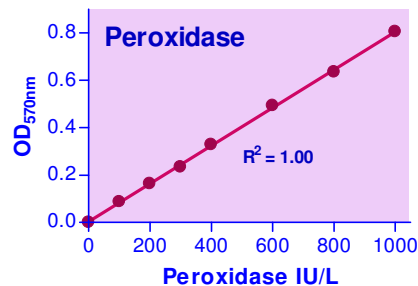
**384-Well Fluorimetric Assay Procedure.** Use 5  $\mu\text{L}$   $\text{H}_2\text{O}$ , 5  $\mu\text{L}$  sample, 35  $\mu\text{L}$  Working Reagent prepared from 38  $\mu\text{L}$  Assay Buffer, 0.2  $\mu\text{L}$  Dye Reagent, 0.2  $\mu\text{L}$  0.6%  $\text{H}_2\text{O}_2$ . Use 40  $\mu\text{L}$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

#### GENERAL CONSIDERATIONS

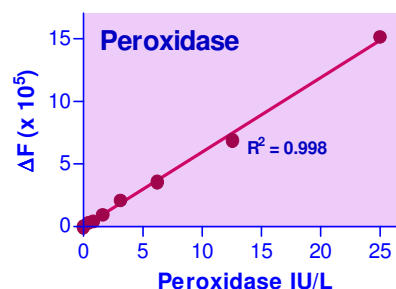
This assay is based on a kinetic reaction, the use of a multi-channel pipettor for adding the Working Reagent and Stop Reagent is recommended.

#### MATERIALS REQUIRED, BUT NOT PROVIDED

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.



96-well Colorimetric Assay



384-well Fluorimetric Assay

#### LITERATURE

1. Kokkinakis DM, Brooks JL. (1979). Tomato Peroxidase: Purification, Characterization, and Catalytic Properties. *Plant Physiol.* 63(1):93-99.
2. Pettigrew GW, Seilman S. (1982). Purification and properties of a cross-linked complex between cytochrome c and cytochrome c peroxidase. *Biochem J.* 201(1):9-18.
3. Smith AL, et al. (1974). Brain polymorphonuclear leukocyte quantitation by peroxidase assay. *Infect Immun.* 10(2):356-60.