Technical Manual

ColorFluor Peroxidase Activity Assay Kit

Catalogue Code: BA0008

Pack Size: 100 assays

Research Use Only

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DESCRIPTION

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. The Assay Genie Peroxidase Activity Assay kit uses H_2O_2 and an electron donor dye that forms resorufin during the peroxidase reaction. The optical density (570 nm) or fluorescence intensity ($\lambda_{ex/em}$= 530/585 nm) is a direct measure of the enzyme activity.

KEY FEATURES

Safe. Non-radioactive assay.

Sensitive and accurate. Use as little as 10 µL samples. Linear detection range: colorimetric assays 2 to 50 U/L, fluorimetric assays 0.1 to 5 U/L peroxidase.

Convenient and high-throughput. "Mix-incubate-measure" type assay. Can be readily automated with HTS liquid handling systems.

APPLICATIONS

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

KIT CONTENTS

Assay Buffer:	20 mL	**Stop Reagent:**	12 mL	**Resorufin:**	1.5 mL
Dye Reagent:	60 µL	**3% Stabilized H_2O_2:**		100 µL	

Storage conditions: 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.

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.

ASSAY 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% 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}.

Fluorimetric Procedure

For fluorimetric 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.

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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).

DATA ANALYSIS

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 fluorimetric 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*.

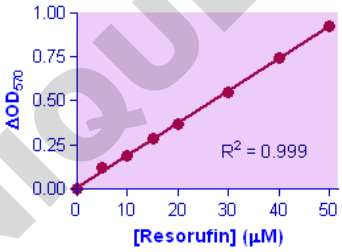
Unit definition: one unit of enzyme will catalyze the formation of 1 µmole resorufin per min under the assay conditions.

GENERAL CONSIDERATIONS

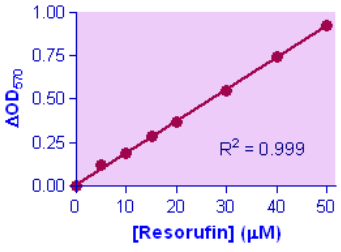
This assay is based on a kinetic reaction, the use of a multi-channel pipettor for adding the Working Reagent and Stop Reagent is recommended.

MATERIALS REQUIRED, BUT NOT PROVIDED

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.



96-well Colorimetric Assay



96-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.

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