Technical Bulletin

Peroxidase Assay Kit

Catalogue number MAK506

Product Description

Peroxidases catalyze the following oxidation-reduction reactions:

ROOR' + electron donor ($2e^-$) + 2H+ → 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 (for example myeloperoxidase-like enzymes), plants, 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. The Peroxidase 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 at λ_{Ex} =530 nm/λ_{Em} =585 nm is a direct measure of the enzyme activity.

The linear detection range of the kit is 2 to 50 U/L peroxidase for colorimetric assays and 0.1 to 5 U/L for fluorometric assays. The kit is suitable for Peroxidase activity determination in biological samples such as plasma, serum, urine, tissue, and culture media.

Components

The kit is sufficient for 100 colorimetric/fluorometric assays in 96-well plates.

- Assay Buffer 20 mL
  Catalogue Number MAK506A
- Stop Reagent 12 mL
  Catalogue Number MAK506B
- Resorufin 1.5 mL
  Catalogue Number MAK506C
- Dye Reagent 60 µL
  Catalogue Number MAK506D
- 3% Stabilized H_2O_2 100 µL
  Catalogue Number MAK506E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example., multichannel pipettor)
- Multiwell plate reader.
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

H_2O_2 Reagent: Dilute 3% H_2O_2 in Assay Buffer to 0.6% and use within one hour. Use freshly diluted 0.6% H_2O_2.

Note: 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.

Procedure

All Samples and Standards should be run in duplicate.

It is prudent to test multiple sample dilutions to ensure activity is in the linear range.

Working Reagent Preparation

Mix enough reagents for the number of assays to be performed. For each well, prepare 96 µL of Working Reagent according to Table 1.

Table 1.
Preparation of Working Reagent

Reagent	Volume
Assay Buffer	95 µL
Dye reagent	0.5 µL
H_2O_2 (0.6%)	0.5 µL

Colorimetric Assay

1. Add 100 µL purified water and 100 µL Resorufin into two wells of a clear flat bottom 96-well plate.
2. Transfer 10 µL purified water (sample blank), 10 µL sample to separate wells.
3. Add 90 µL Working Reagent into each well. Tap plate to mix.
4. Incubate the plate for 10 minutes at room temperature.
5. Add 100 µL Stop Reagent to all wells. Read the optical density at 570nm.

Fluorometric Procedure

1. Dilute the Resorufin 1:10 in purified water.
2. Resorufin into two wells of a black flat-bottom 96-well plate.
3. Transfer 10 µL purified water and 10 µL sample to separate wells.
4. Add 90 µL Working Reagent into each well. Tap plate to mix.
5. Incubate the plate for 10 minutes at room temperature.
6. Add 100 µL Stop Reagent to all wells. Tap plate to mix. Read fluorescence ($\lambda_{ex}/\lambda_{em}$= 530/585 nm).

Note: 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.

Results

The peroxidase activity in a sample is computed as follows:

Peroxidase activity

$$= \frac{R_{Sample}-R_{Blank}}{R_{Resorufin}-R_{H2O}} \times \frac{[Resorufin](\mu M)}{t\ (min)} \times \frac{Reaction\ vol\ (\mu L)}{Sample\ vol\ (\mu L)} \times n$$

$$= \frac{R_{Sample}-R_{Blank}}{R_{Resorufin}-R_{H2O}} \times [Resorufin](\mu M) \times n\ (U/L)$$

Where:

R_{Sample}, R_{Blank}, $R_{Resorufin}$ and R_{H2O} are optical density or fluorescence readings of the Sample, Sample Blank, Resorufin and Water respectively.

n is the dilution factor.

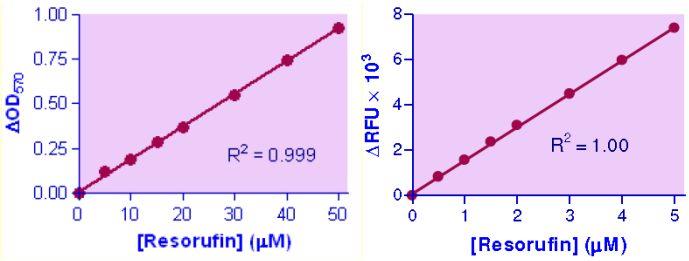
The [Resorufin] is 50 µM for colorimetric assays and 5 µM for fluorometric assays.

The Reaction Vol is 100 µL and the Sample Vol is 10 µL.

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

Figure 1.

Example of Resorufin standard curves by colorimetric and fluorometric assay.



96-well Colorimetric Assay *96-well Fluorimetric Assay*

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