Peroxidase Activity Assay Kit (Colorimetric)

Peroxidase Activity Assay Kit (Colorimetric) can be used to measure Peroxidase activity in serum, plasma, urine, tissue and cell culture supernatants.

Catalog number: ARG82188

Package: 100 tests

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INTRODUCTION

Peroxidases or peroxide reductases (EC number 1.11.1.x) are a large group of enzymes which play a role in various biological processes. They are named after the fact that they commonly break up peroxides.

Peroxidases typically catalyze a reaction of the form:

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

[Provide by Wikipedia: Peroxidase]

PRINCIPLE OF THE ASSAY

This Peroxidase Activity Assay Kit (Colorimetric) is a simple assay that measures the activity of Peroxidase in various biological samples such as serum, plasma, tissue and cell culture supernatant. This assay uses H_2O_2 and an electron donor dye that forms resorufin during the peroxidase reaction. The optical density (O.D. 570 nm) or fluorescence intensity (λex/em = 530/585 nm) is a direct measure of the enzyme activity.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped at room temperature. Store all components at -20°C upon receiving. Shelf life: 12 months after receipt.

Component	Quantity	Storage information
Assay Buffer	20 mL	-20°C
3% Stabilized H_2O_2	100 µL	-20°C
Dye Reagent	60 µL	-20°C
Stop Reagent	12 mL	-20°C
Standard (50 µM Resorufin)	1.5 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570 nm
- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm
- Centrifuge and centrifuge tube
- Clear or black flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collection the supernatant for assay.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collection the supernatant for assay.

Tissue or cell lysate: Tissue or cell samples (2 x10^6) can be homogenized in 100 µL of PBS. Centrifuge at 10,000 x g for 5 minutes at 4°C. Collection the supernatant for assay.

Cell culture supernatant: Centrifuge at 1000 x g for 10 minutes. Collection the supernatant for assay.

REAGENT PREPARATION

- **0.6% H_2O_2**: Dilute 3% H_2O_2 in Assay Buffer to 0.6% H_2O_2 and use within one hour. (E.g., add 4 µL of 3% H_2O_2 into 16 µL of Assay Buffer.)
- **Working Reagent:** for each assay, mix 95 µL of Assay Buffer, 0.5 µL of Dye Reagent and 0.5 µL of fresh diluted 0.6% H_2O_2. Prepare immediately before assay.

ASSAY PROCEDURE

Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening.

COLORIEMTRIC PROCEDURE

	Standard well	Sample well	Blank well	H_2O well
Standard	100 µL			
Distilled water			10 µL	100 µL
Each Sample		10 µL		
Working Reagent		90 µL	90 µL	
Tap plate to mix briefly and thoroughly. Incubate for **10 minutes** at **room temperature**.				
Stop Reagent	100 µL	100 µL	100 µL	100 µL
Tap plate to mix briefly and thoroughly. Read the absorbance at **O.D. 570 nm**.				

FLUORIMETRIC PROCEDURE

Follow COLORIEMTRIC PROCEDURE with the change below. The linear detection range is 0.1 to 5 U/L. Dilute the Standard (Resorufin) 1:10 in distilled water. Use black flat-bottom 96 well microplate for assay. And read the fluorescence at λex/em = 530/585 nm.

CALCULATION OF RESULTS

1. The peroxidase activity in a sample is calculated as follow:

   Peroxidase Activity (U/L)

   = $[(R_{Sample} - R_{Blank}) / (R_{Standard} - R_{H2O})]$ x [Standard (µM) / t (min)] x (Reaction Vol / Sample Vol) x n

   = $[(R_{Sample} - R_{Blank}) / (R_{Standard} - R_{H2O})]$ x Standard (µM) x n

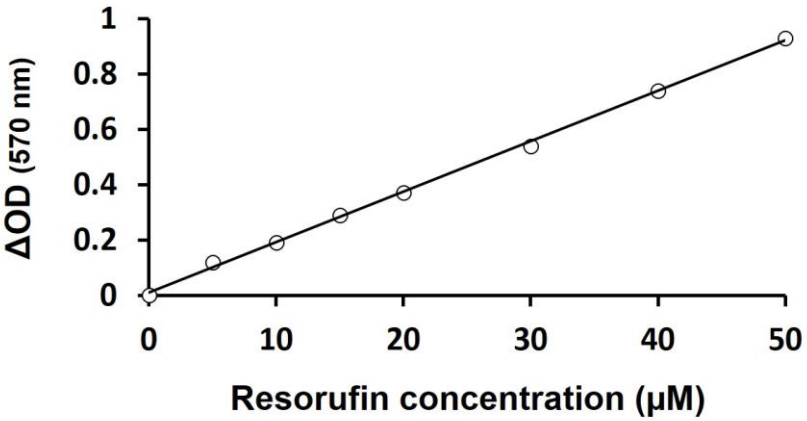
   Note:

   - R_{Sample}, R_{Blank}, $R_{Standard}$ and R_{H2O}: the O.D. 570 nm values and fluorescence intensity values of the Sample, Blank, Standard (Resorufin) and H_2O well, respectively.
   - The Standard (µM) 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.
   - n is the sample dilution factor.

2. Unit definition: one unit of enzyme will catalyze the formation of 1 µmole resorufin per min under the assay conditions.
3. If Sample O.D. or fluorescence values are higher than that of the Standard (Resorufin), dilute sample in Assay Buffer, repeat assay and multiply results by the dilution factor, n.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Peroxidase Activity Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

4 U/L (colorimetric); 0.8 U/L (fluorometric)