MANUAL

Peroxidase Activity Assay Kit (Colorimetric)

Catalog #: MA-POD

ISO 13485:2016

Introduction

Peroxidases, a group of heme-containing enzymes, catalyze oxidation-reduction reactions. They utilize hydrogen peroxide (H_2O_2) or lipid peroxides as substrates for various oxidative reactions. Peroxidases are present in bacteria, fungi, plants, and animals, and they play a crucial role in protecting cells from oxidative damage.

Peroxidase Activity Assay Kit offers a straightforward, reproducible, and highly sensitive method for measuring peroxidase activity in plasma, serum, cell lysates, urine, and other biological liquid samples without requiring any pre-treatment. In this assay, an ultra-sensitive colorimetric probe reacts with both hydrogen peroxide (H_2O_2) and peroxidase enzyme to generate a pink-colored product. The intensity of the pink color is then quantified at 570nm, providing a direct measure of the peroxidase content in the sample.

Storage

The entire kit may be stored at < -20 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate (Item A)	A 96-well (12 strips x 8 wells) plate	RT*
Assay Buffer	25 ml	2–8 °C, 4 weeks
Peroxidase (Horseradish Peroxidase) Standard	1 amber vial (Lyophilized)	Do not store and reuse
Hydrogen Peroxide	1 vial (20 µl of 8.82M)	Do not store and reuse
Ultra Colorimetric Probe	1 amber vial	Aliquot and store at -20°C

RT = room temperature

**Return unused wells to the pouch containing desiccant pack, reseal along entire edge.*

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 570 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Multi-channel pipettes to deliver 20 µl to 200 µl volumes
4. Tubes to prepare sample dilutions
5. 15 ml conical tubes

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquotted and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. Samples may be assayed directly or diluted as necessary in Assay Buffer.

3. Serum samples:

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. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Samples may be assayed directly or diluted as necessary in Assay Buffer.

4. Urine samples:

To remove insoluble particles, spin at 10,000 x g for 5 min. The supernatant can be assayed directly.

Note:
Optimal experimental conditions for samples must be determined by the investigator. A set of serial dilutions is recommended for samples to achieve optimal assay results.

If the calculated peroxidase activity of the sample is higher than 10 U/L, dilute the sample in assay buffer and repeat the assay.

Reagent Preparation

REAGENT	PREPARATION
Microplate (Item A)	No preparation
Assay Buffer	
Peroxidase (Horseradish Peroxidase) Standard	See "Standard Preparation" section.
Hydrogen Peroxide	Add 3 µL of 8.8M H_2O_2 (provided) into 2997 µL Assay Buffer to get 8.8mM H_2O_2. (*Note:* Diluted Hydrogen Peroxide is unstable and should be kept on ice while being used. Do not store diluted Hydrogen Peroxide solutions.)
Ultra Colorimetric Probe	Warm to room temperature before use.
Chromogenic Working Solution	Add 69 µL of 8.8mM H_2O_2 (see preparation above) and 15 µL Ultra Colorimetric Probe into 2916 µL Assay Buffer to make Chromogenic Working Solution. This is enough to assay 60 wells. Prepare the additional vial as needed. The solution is stable for 1 day at 2-8°C in the dark.

Standard Preparation

To prepare a dilution series of standard in the concentration range of 0 U/L – 10 U/L (see Table below):

1. Label 8 microtubes #1 through #8 for the following concentrations: 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625, 0 U/L.
2. Pipette appropriate volume of Assay Buffer into labeled tube #1 through #8 according to the table below.
3. To make 500 U/L Horseradish Peroxidase (HRP) standard stock: Briefly spin down the Peroxidase (Horseradish Peroxidase) Standard tube, pipette 50 µL of Assay Buffer into this tube to get 500 U/L HRP standard stock.
4. Pipette 20 µL of 500 U/L HRP standard stock into tube #1, to make 10 U/L HRP standard.
5. To make the 5 U/L standard, pipette 200 µL of the tube #1 into the tube labeled #2. Mix thoroughly.
6. Repeat this step with successive concentration, preparing a dilution series as shown in the Table below. Mix each tube thoroughly before the next transfer. Standards should be prepared freshly, mixed thoroughly and used immediately.

Labeled Tubes	Horseradish Peroxidase Standard (µL)	Assay Buffer (µL)	Standard Activity (U/L)
1	20 µL of 500U/L HRP	980 µL	10
2	200 µL of Tube #1	200 µL	5
3	200 µL of Tube #2	200 µL	2.5
4	200 µL of Tube #3	200 µL	1.25
5	200 µL of Tube #4	200 µL	0.625
6	200 µL of Tube #5	200 µL	0.3125
7	200 µL of Tube #6	200 µL	0.15625
8	0 µL	200 µL	0

Assay Procedure

Each peroxidase standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

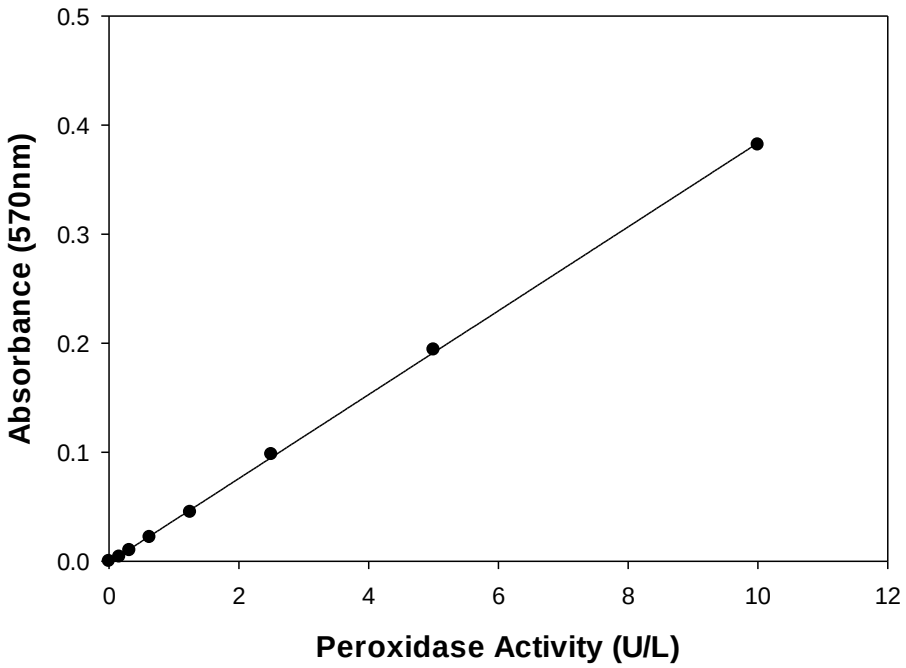
1. Add 50 µL of the diluted Horseradish Peroxidase standards or unknown samples to each well of the Microplate.
2. Add 50 µL of the Chromogenic Working Solution (See “Reagent Preparation”) to each well. Incubate the plate at room temperature for 30 minutes gently mixing in the dark.
3. Measure the absorbance at 570nm using a plate reader.

Calculation of Results

1. Calculate the average absorbance values for each set of standards and samples.
2. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit curve through the standard points.
3. Use the mean absorbance value for each sample to determine the corresponding concentration from the standard curve.

Note: If the calculated peroxidase activity of the sample is higher than 10 U/L, dilute the sample in assay buffer and repeat the assay.

A. Typical Data



These standard curves are for demonstration only. A standard curve must be run with each assay.

B. Sensitivity

The minimum detectable peroxidase activity is 0.006 U/L.

C. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 1.1%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 1.4%.

This product is for research use only.