Peroxidase Assay Kit

Catalog No.: abx298828

Size: 100 Assays

Storage: Store the Positive Control at -20°C and all other kit components in the dark at 4°C.

Application: For quantitative detection of Peroxidase activity in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, and other biological fluids.

Introduction: Peroxidases are enzymes found in many biological systems that catalyze redox reactions, often acting on hydrogen peroxide as the substrate. They play an important role in protecting cells from oxidative damage.

The concentration of the reaction product generated by this assay can be determined by measuring the absorbance at 470 nm, from which the enzyme activity can be calculated.

Kit Components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 5 ml
4. Substrate: 10 ml
5. Dye Reagent: 4 ml
6. Positive Control: 1 vial

Materials Required But Not Provided

1. Microplate reader (470 nm)
2. Microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Mortar
6. Centrifuge and centrifuge tubes
7. Timer
8. Ice
9. Sonicator

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Positive Control Solution**
  Add 1 ml of distilled water to the Positive Control vial and mix thoroughly. Ensure that the Positive Control has completely dissolved. Add 30 µl of this solution to 970 µl of distilled water and mix thoroughly to prepare the Positive Control Solution.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 8000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice. Centrifuge at 8000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Serum and Plasma samples**
  Serum and plasma samples can be used directly.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Set the sample and positive control wells on the 96 well microplate and record their positions. We recommend setting up each sample in duplicate.
2. Add 10 µl of sample to the sample wells.
3. Add 10 µl of Positive Control Solution to the positive control wells.
4. Add 50 µl of Reaction Buffer to all wells.
5. Add 100 µl of Substrate to all wells.
6. Add 40 µl of Dye Reagent to all wells.
7. Tap the plate gently to mix. Start the timer.
8. Read and record absorbance at 470 nm after 20 seconds and after 140 seconds.

C. Calculations

One unit of Peroxidase activity is defined as the change in OD/0.01 per minute in the reaction system.

Peroxidase activity per mg of protein:

$$\textbf{Peroxidase}\ (\mathbf{U/mg}) = \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample}} \times \mathbf{C_{Protein}} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample(140s)}} - \mathbf{OD_{Sample(20s)}}}{\mathbf{0.01}} = \frac{\mathbf{1000}}{\mathbf{C_{Protein}}} \times \left(\mathbf{OD_{Sample(140s)}} - \mathbf{OD_{Sample(20s)}}\right)$$

Peroxidase activity per g of sample:

$$\textbf{Peroxidase}\ (\mathbf{U/g}) = \frac{\mathbf{V_{Total}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{W} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample(140s)}} - \mathbf{OD_{Sample(20s)}}}{\mathbf{0.01}} = \frac{\mathbf{1000}}{\mathbf{W}} \times \left(\mathbf{OD_{Sample(140s)}} - \mathbf{OD_{Sample(20s)}}\right)$$

Peroxidase activity per 10^4 cells or bacteria:

$$\textbf{Peroxidase}\ (\mathbf{U/10^4\ cells}) = \frac{\mathbf{V_{Total}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{N} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample(140s)}} - \mathbf{OD_{Sample(20s)}}}{\mathbf{0.01}} = \frac{\mathbf{1000}}{\mathbf{N}} \times \left(\mathbf{OD_{Sample(140s)}} - \mathbf{OD_{Sample(20s)}}\right)$$

Peroxidase activity per ml of serum or plasma:

$$\textbf{Peroxidase}\ (\mathbf{U/ml}) = \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample}} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample(140s)}} - \mathbf{OD_{Sample(20s)}}}{\mathbf{0.01}} = \mathbf{1000} \times \left(\mathbf{OD_{Sample(140s)}} - \mathbf{OD_{Sample(20s)}}\right)$$

where:

$\mathbf{OD_{Sample(20s)}}$	Absorbance at 470 nm taken after 20 seconds
$\mathbf{OD_{Sample(140s)}}$	Absorbance at 470 nm taken after 140 seconds
$\mathbf{T}$	Reaction time (2 minutes)
$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{W}$	Weight of the sample (in g)
$\mathbf{N}$	Number of cells or bacteria (× 10^4)
$\mathbf{V_{Assay}}$	Volume of assay buffer used in sample preparation (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (10 µl =0.01 ml)
$\mathbf{V_{Total}}$	Total volume of reaction system (200 µl = 0.2 ml)