Ascorbate Oxidase Assay Kit

Catalog No.: abx298817

Size: 100 Assays

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

Application: For quantitative detection of Ascorbate Oxidase activity in tissue homogenates and cell lysates.

Introduction: Ascorbate Oxidase (AAO) is an enzyme involved in the metabolism of ascorbate in plants. Ascorbate plays a key role in the defense against oxidative stress and is particularly abundant in photosynthetic tissues.

Abbexa's Ascorbate Oxidase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Ascorbate Oxidase activity. The concentration of ascorbic acid (AsA) generated by this assay can be determined by measuring the absorbance at 265 nm, from which the enzyme activity can be calculated.

Kit Components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 20 ml
4. Substrate: 1 vial
5. Positive Control: 1 vial

Materials Required But Not Provided

1. Microplate reader (265 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

- **Substrate Solution**
  Add 1 ml of Reaction Buffer to the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.

- **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 0.5 ml of this solution to 0.5 ml 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 16,000 × g at 4°C for 10 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 16,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

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 180 µl of Reaction Buffer to all wells.
3. Add 10 µl of Substrate Solution to all wells.
4. Add 10 µl of sample to the sample wells.
5. Add 10 µl of Positive Control Solution to the positive control wells.
6. Tap the plate gently to mix. Start the timer.
7. Read and record absorbance at 265 nm after 10 seconds and after 130 seconds.

C. Calculations

One unit of Ascorbate Oxidase activity is defined as the amount of enzyme required to oxidize 1 µmol of ascorbic acid per minute.

Ascorbate Oxidase activity per mg of protein:

$$\textbf{Ascorbate Oxidase}\ (\mathbf{U/mg}) = \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample}} \times \mathbf{C_{Protein}} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Sample(10s)}}}{\varepsilon \times \mathbf{d}} = \frac{\mathbf{0.308}}{\mathbf{C_{Protein}}} \times \left(\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Sample(10s)}}\right)$$

Ascorbate Oxidase activity per g of sample:

$$\textbf{Ascorbate Oxidase}\ (\mathbf{U/g}) = \frac{\mathbf{V_{Total}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{W} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Sample(10s)}}}{\varepsilon \times \mathbf{d}} = \frac{\mathbf{0.308}}{\mathbf{W}} \times \left(\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Sample(10s)}}\right)$$

Ascorbate Oxidase activity per 10^4 cells or bacteria:

$$\textbf{Ascorbate Oxidase}\ (\mathbf{U/10^4\ cells}) = \frac{\mathbf{V_{Total}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{N} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Sample(10s)}}}{\varepsilon \times \mathbf{d}} = \frac{\mathbf{0.308}}{\mathbf{N}} \times \left(\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Sample(10s)}}\right)$$

where:

$\mathbf{OD_{Sample(10s)}}$	Absorbance at 265 nm taken after 10 seconds
$\mathbf{OD_{Sample(130s)}}$	Absorbance at 265 nm taken after 130 seconds
$\boldsymbol{\varepsilon}$	Molar extinction coefficient (5.42×10^4 L /mol /cm = 54.2 ml /µmol /cm)
$\mathbf{d}$	Optical path for a 96-well microplate (0.6 cm)
$\mathbf{T}$	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 ($\times 10^4$)
$\mathbf{V_{Assay}}$	Volume of assay buffer (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)