Glycolate Oxidase Assay Kit

Catalog No.: abx298869

Size: 100 Assays

Detection Range: 0.3 U/ml – 350 U/ml

Sensitivity: 0.3 U/ml

Storage: Store all components in the dark at 4°C.

Application: For detection and quantification of Glycolate Oxidase activity in tissue homogenates.

Introduction

Glycolate Oxidase is an enzyme found in plant cell peroxisomes, and catalyses the oxidation of glycolic acid to glycoxylic acid. Glycolate Oxidase is a key enzyme in plant photorespiration, and can be used to indirectly measure photorespiration in plants.

Abbexa's Glycolate Oxidase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Glycolate Oxidase activity. The reaction products have an absorbance maxima at 324 nm. The intensity of the color is proportional to the Glycolate Oxidase activity, which can then be calculated.

Kit components

1. Substrate Solution: 12 ml
2. Buffer Solution: : 2 × 45 ml
3. Extraction Solution: 2 × 60 ml
4. Detection Reagent: 1 vial

Materials Required But Not Provided

1. Spectrophotometer (324 nm, 1 cm optical path cuvette)
2. Double distilled water
3. Pipette and pipette tips
4. Homogenizer
5. Vials/tubes
6. Centrifuge
7. Vortex mixer
8. Timer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **10% Tissue Homogenates:** Accurately weigh 0.1 g of tissue and wash with 0.9 ml of pre-cooled extraction solution. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication on ice. Centrifuge the homogenate at 12,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% *Oxalis corniculata* Tissue Homogenate	1
10% *Ginkgo biloba* Tissue Homogenate	1
10% Bamboo leaf Tissue Homogenate	1
10% Cactus Tissue Homogenate	1
10% *Osmanthus fragrans* leaf Tissue Homogenate	4-8
10% Camphor tree leaf Tissue Homogenate	3-5

Note:

- Where dilutions are required, samples should be diluted with Extraction Solution.
- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Detection Reagent working solution:** Dissolve a vial of Detection Reagent with 24 ml double distilled water and mix fully. Prepare immediately before assay, or aliquot and store at -20°C in the dark. Avoid repeated freeze-thaw cycles.

B. Assay Procedure

1. Set blank and sample tubes and label accordingly.
2. Add 50 µl of double distilled water to the blank tube.
3. Add 50 µl of sample to each of the sample tubes.
4. Add 650 µl of Buffer Solution to all tubes.
5. Add 200 µl of Detection Reagent working solution to all tubes.
6. Add 100 µl of Substrate Solution to all tubes.
7. Mix all tubes fully. Calibrate the spectrophotometer to zero with double distilled water (324 nm, 1 ml volume, 1 cm optical path).
8. Measure and record the OD with the spectrophotometer (A_1). Begin the timer. *Note: if the OD is greater than 1.0, the sample should be diluted.*
9. After exactly 3 minutes, measure and record the OD with the spectrophotometer (A_2).

C. Calculation of Results

Tissues samples:

One unit of Glycolate Oxidase activity is defined as the quantity of Glycolate Oxidase in 1 mg of tissue required to oxidize 1 nmol of glycolic acid at room temperature in 1 minute.

$$\text{Glycolate oxidase activity (U/mg)} = \frac{(\mathrm{A_2} - \mathrm{A_1}) - (\mathrm{A_{Blank\,2}} - \mathrm{A_{Blank\,1}})}{\varepsilon \times d} \times \frac{1000 \times f \times 10^3}{50 \times T \times \mathrm{C_{Protein}}}$$

where:

$\mathrm{A_2}$	OD of sample at 3 minutes
$\mathrm{A_1}$	OD of sample at the beginning of the reaction
$\mathrm{A_{Blank\,2}}$	OD of blank at 3 minutes
$\mathrm{A_{Blank\,1}}$	OD of blank at the beginning of the reaction
ε	Molar extinction coefficient of phenylhydrazone glyoxylate (17 L/mmolcm)
d	Optical path of the cuvette (1 cm)
f	The dilution factor of sample
1000	Total volume of the reaction (1000 µl)
10^3	Conversion (1 µmol = 1000 nmol)
50	Volume of sample added to the reaction (50 µl)
T	Reaction time (3 minutes)
$\mathrm{C_{Protein}}$	Concentration of protein in sample