PRODUCT INFORMATION & MANUAL

Glycolate Oxidase Activity Assay Kit (Colorimetric)

NBP3-24503

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Glycolate Oxidase Activity Assay Kit

Catalog No: NBP3-24503

Method: Colorimetric method

Specification: 100 Assays(Can detect 98 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.3 U/ml

Detection range: 0.3-350 U/ml

Average intra-assay CV(%): 1.1

Average inter-assay CV(%): 8.2

Average recovery rate(%): 102

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

_. Intended use

This kit can be used to measure glycolate oxidase activity in plant tissue sample.

_. Background

Glycolate oxidase, a non-mitochondrial oxidase, is found in plant cell peroxidasomes and can catalyze the oxidation of glycolic acid to glyoxylic acid and release hydrogen peroxide. Glycolate oxidase is one of the key enzymes in plant photorespiration, and its activity can be used as an important index to measure the intensity of plant photorespiration, which changes with plant development, mineral nutrition and disease susceptibility.

_. Detection principle

Glycolate oxidase catalyzes sodium glycolate substrate to form glyoxylic acid, which reacts with phenylhydrazine hydrochloride to form phenylhydrazone glyoxalate. The substance has an absorption peak at 324 nm, and its OD value is proportional to the concentration of phenylhydrazone glyoxalate in a certain range, and the amount of phenylhydrazone generated reflects the activity of glycolate oxidase.

_. Kit components & storage

Item	Component	Specification	Storage
Reagent1	Extraction Solution	60 ml x2 vials	2-8°C, 12 months
Reagent2	Buffer Solution	45 ml x2 vials	2-8°C, 12 months, shading light
Reagent3	Chromogenic Agent	Powder x1 vial	2-8°C, 12 months, shading light
Reagent4	Substrate	12 ml x1 vial	2-8°C, 12 months
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

_. Materials prepared by users

Instruments

Test tube, Micropipettor, Vortex mixer, Spectrophotometry (324 nm), Optical path cuvette (1 ml of volume, 1 cm optical diameter).

Reagents

Double distilled water

Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

The key points of the assay

1. When the OD value is more than 1.0, the sample must be diluted.
2. If use a mortar to prepare tissue homogenate, precool the mortar at a 4 °C environment.

Pre-assay preparation

Reagent preparation

1. Bring all reagents to room temperature before use except reagent 1 which needs to be on ice box for detection.
2. Preparation of working solution:
   Dissolve the reagent 3 with 24 ml of purified water and mix fully. The prepared solution can be aliquoted into small qualities and stored at -20˚C with shading light to avoid repeated freezing and thawing.

Sample preparation

Tissue sample: Accurately weigh 0.1 g plant tissue sample, add 0.9 ml of pre-cooled reagent 1. Mechanical homogenate the sample at 4 ˚C. Centrifuge at 12000 g at 4 ˚C for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the supernatant sample can be stored at -20˚C for 2 days.

Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.3-350 U/ml).

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% Cactus tissue homogenate	1
10% Bamboo leaf tissue homogenate	1
10% Osmanthus fragrans leaf tissue homogenate	4-8
10% Camphor trees leaf tissue homogenate	3-5

Note: The diluent is reagent 1.

Assay protocol

_. Detailed operating steps

1) Blank tubes: Take 50 µl of double distilled water to 5 ml EP tube.
   Sample tubes: Take 50 µl of sample to 5 ml EP tube.
2) Add 650 µl of reagent 2, 200 µl of working solution and 100 µl of reagent 4 to each tube.
3) Mix fully and set to zero with double distilled water and measure the OD value of each tube with 1 ml of volume and 1 cm optical path cuvette at 324 nm, recorded as A_1.
4) Stand for 3 min and detect again, recorded as A_2.

_. Summary operation table

	Blank tube	Sample tube
Double distilled water (µl)	50	
Sample (µl)		50
Reagent 2 (µl)	650	650
Working solution (µl)	200	200
Reagent 4 (µl)	100	100
Mix fully and measure the OD value at 324 nm, recorded as A_1. Stand for 3 min and detect again, recorded as A_2.		

Calculation

Definition: the amount of enzyme in 1 mg of tissue protein that oxidize 1 nmol glycollic acid at room temperature for 1 min is defined as 1 activity unit.

Glycolate oxidase activity (U/mgprot) = /iA + (e xd) +50 x1000 x f x10^3+T+ Cpr

Note:

liA: liA = ($_2$ -A_1) - (A_{02} - A_{01})

A_1: The OD value of initial sample tube;

A_2: The OD value of sample tube after 3 min;

A_{01}: The OD value of initial blank tube;

A_{02}: The OD value of blank tube after 3 min;

e: The molar extinction coefficient of phenylhydrazone glyoxylate, 17 L/(mmol•cm);

d: The optical path of cuvette, 1 cm;

50: The volume of sample added to the reaction, 50 μL;

1000: The total volume of reaction, 1000 μL;

f: The dilution multiple of tested samples;

T: Reaction time, 3 min;

Cpr: The concentration of protein in sample, mgprot/ml;

10^3: 1 μmol = 1000 nmol.

Appendix I Data

Example analysis

For oxalis corniculata, take 50 μL tissue supernatant and carry the assay according to the operation table. The results are as follows:

The A_{01} of the blank is 0.008, the A_{02} of the blank is 0.010, the A_1 of the sample is 0.577, the A_2 of the sample is 0.608, the concentration of protein in sample is 8.93 mgprot/ml, and the result is:

/iA = (0.608 -0.577) - (0.010 -0.008) = 0.029

Glycolate oxidase activity (U/mgprot)

= 0.029 + (17 x1) +50 x1000 x10^3+3+ 8.93=1.27 U/mgprot

Detect 10% oxalis corniculata tissue homogenate (the concentration of protein is 8.93 mgprot/ml), 10% ginkgo blioba tissue homogenate (the concentration of protein is 3.04 mgprot/ml), 10% epipremnum aureum tissue homogenate (the concentration of protein is 5.39 mgprot/ml) and 10% rassula arborescens tissue homogenate (the concentration of protein is 5.97 mgprot/ml) according to the protocol, the result is as follows:

Appendix II References

1. Li M. Plant Glycolate Oxidase. Plant Physiology Communication, 1988(6): 67-71.
2. Zhou J. Effected of Heat Stress on Leaf Proteome and Enzyme Activity in Solanum chilense. Global Science Books, 2012.6(1): 8-13.