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Glycolate oxidase (GO) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/ microplate reader

Cat No: AK0252

Size: 100T/96S

Components:

Extract solution: Liquid 60 mL×2, store at 4℃;

Reagent I: Liquid 15 mL×1, store at 4℃;

Reagent II: Powder×2, store at -20℃ . Add 2.5 mL distilled water when the solution will be used. The rest of reagent store at -20℃ . Do not freeze and thaw repeatedly;

Reagent III: Liquid 2 mL×1, store at 4℃ and protect from light.

Product Description:

Glycolate oxidase (EC1. 1.3. 15) is an enzyme in the glycolate cycle. It is also a key enzyme in plant photorespiration metabolism. It can catalyze the oxidation of glycolic acid to glyoxylic acid. By measuring the activity of glycolate oxidase, we can understand the basic methods of plant photosynthesis and respiratory metabolism.

Glycolate oxidase catalyzes the oxidation of glycolic acid to glyoxylic acid. Glyoxylic acid reacts with phenylhydrazine hydrochloride to form phenylhydrazone glyoxylate. There is a characteristic absorption peak at 324 nm.

Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, low temperature desk centrifuge, balance, transferpettor, mortar/homogenizer, ice, micro quartz cuvette/96 well UV plate and distilled water.

Protocol

I. Preparation:

1. Tissue: according to the ratio of mass (g): Extract solution volume (mL): 1:5- 10 to add the extract. It is suggested that add 1 mL of extract to 0.1 g of tissue. Homogenate on ice. Centrifuge at 10000 g 4℃ for 10 min. Take the supernatant on ice for test.

2. Cells: according to the number of the cells (10^4): the volume of the Extract solution (mL) is 500~1000:1. It is suggested that add 1 mL of extraction reagent to 500 million of cells. Breaking cells by ultrasonic wave in ice bath (power 300W, ultrasonic 3s, interval 7s, total time 3 min). Centrifuge at 10000 g 4℃ for 10 min. Take the supernatant on ice for test.

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 324 nm, set the counter to zero with distilled water.

2. Preheat reagent I in 25℃ for 15 min.

3. Operation table:

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)
Reagent I	130	130
Distilled water	-	10
Sample	10	-
Reagent II	40	40
Reagent III	20	20
Mix thoroughly. Measure the absorbance A1 for 10s and the absorbance A2 for 190s at 340 nm. $\Delta A_B=A2_B-A1_B$. $\Delta A_T=A2_T-A1_T$. $\Delta A=\Delta A_T-\Delta A_B$. Blank tube only needs to test once or twice.		

III. GO Calculation:

a. Micro quartz cuvette

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol phenylhydrazine glyoxylate in the reaction system per minute every mg protein.

GO (U/mg prot) $=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(Cpr\times V_{SA})\div T\times 10^9= 392.16\times\Delta A\div Cpr$

2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol phenylhydrazine glyoxylate in the reaction system per minute every g sample.

GO (U/g weight) $=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(W\div V_E\times V_{SA})\div T\times 10^9=392.16\times\Delta A\div W$

3) Cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol phenylhydrazine glyoxylate in the reaction system per minute every 10^4 cells.

GO (U/10^4 cell) $=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(500\times V_{SA}\div V_E)\div T\times 10^9=0.784\times\Delta A$

ε: Phenylhydrazine glyoxylate molar extinction coefficient, 17000 L/mol/cm;

d: Light path of cuvette, 1 cm;

V_{RT}: Total reaction volume, 0.0002 L;

V_{SA}: Sample volume, 0.01 mL;

V_E: Extract solution volume of cells, 1 mL;

500: Cells, 5 million;

T: Reaction time, 3 min;

Cpr: Protein concentration, mg/mL;

10^9: Unit conversion factor, 1 mol = 10^9 nmol.

b. 96 well UV plate

The optical diameter d=1 cm of the cuvette in the above formula is changed to 0.6 cm of the 96 well UV plate.

Note:

1. Carry out pre experiment before determination. If the absorbance value A1＞1, please dilute the sample with the extraction solution for appropriate re determination. And multiply the dilution ratio in the calculation formula.
2. The samples with high pigment content can be adsorbed with active carbon during enzyme extraction.
3. The blank tube is a test tube for testing the quality of each reagent component. Under normal conditions, its OD value does not change more than 0.02.

Experimental example:

1. Weigh about 0. 1g of clover tissue, add 1 mL of Extract solution, homogenize it in ice bath, then centrifuge it at 4℃ for 10 min, take the supernatant for detection, and use micro quartz colorimetric plate to measure the ΔA_T= $A2_T$-$A1_T$=0.8956-0.7839=0. 1117, ΔA_B= $A2_B$-$A1_B$=0.0853-0.077 = 0.0083, ΔA = ΔA_T- ΔA_B= 0. 1117-0.0083 = 0. 1034, according to the sample mass The enzyme activity is calculated as follows

GO activity (U/g mass) = 392.16 × Δ A ÷ W = 405.4934 U/g mass.

2. Weigh about 0. 1g of spinach tissue, add 1 mL of Extract solution, homogenize it in ice bath, then centrifuge it at 4℃ for 10 min, dilute the supernatant twice for detection, and use micro quartz colorimetric plate to measure the ΔA_T= $A2_T$- $A1_T$= 0.9286-0.8105 = 0. 1181, ΔA_B= $A2_B$-$A1_B$= 0.0853-0.077 = 0.0083, ΔA = ΔA_T - ΔA_B= 0. 1181-0.0083 = 0.1098

GO activity (U/g mass) = 392.16 × ΔA÷ W × 2 (dilution ratio) = 861.1834 U/g mass.

Related Products:

AK0246/AK0245 Isocitrate Lyase(ICL) Activity Assay Kit
AK0100/AK0099 4-Coumarate CoA Ligase(4CL) Activity Assay Kit