

Glucose Oxidase (GOD) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Cat No: NA0814

Size: 50T/48S

Components:

Extract solution: Liquid 60 mL×1, store at 4°C.

Reagent I: Liquid 40 mL×1, store at 4°C.

Reagent II: Liquid 8 mL×1, store at 4°C.

Reagent III: Liquid 1 mL×2, store at -20°C. Divide into several vials and save after melting.

Description:

Glucose oxidase (GOD, EC 1.1.3.4) is widely exist in animals and plants. It can catalyze glucose to form gluconic acid and H₂O₂. GOD is one of the metabolic pathways of producing reactive oxygen in organisms.

When glucose is catalyzed to produce H₂O₂ by GOD. Then peroxidase catalyzes the production of H₂O₂ into oxygen, which oxidize o-dianisidine to form colored substance. The color depth is linear with glucose oxidase activity.

Required but not provided:

Spectrophotometer, water-bath, centrifuge, adjustable pipette, 1 mL glass cuvette, mortar/homogenizer, ice and distilled water.

Protocol:

I. Sample Preparation.

1. Tissue: Homogenize 0.1g of tissue sample in 1mL of Extract solution in ice bath. Centrifuge at 8000 ×g for 10 minutes at 4°C and take the supernatant and put it on ice for test.
2. Bacteria and cells: first collect bacteria or cells into the centrifuge tube, then discard the supernatant after centrifugation, add 1 mL of Extract solution to every 5 million bacteria or cells, and break them by ultrasonic wave (power 20% or 200W, ultrasonic wave 3s, interval 10s, repeat 30 times). After centrifugation at 4°C for 10 minutes, the supernatant is taken and placed on ice for testing.
3. Serum: Detect directly.

II. Determination procedure.

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 500 nm and set zero with distilled water.
2. Working solution: Mix thoroughly 20 mL of Reagent I with 4 mL of Reagent II (prepare when the solution will be used)

3. Add reagents according to the following table.

| Reagent name (μL) | Test tube |
|--|-----------|
| Working solution | 870 |
| Reagent III | 30 |
| Mix thoroughly, incubate at 37°C (mammals) or 25°C (other animals) water bath for 5 minutes. | |
| Sample | 100 |

Incubate working solution and Reagent III at 37°C water bath before testing. Then the above reagents are added to the 1 mL glass cuvette in order, and the timing begin at the same time as the samples are added. Then the initial absorbance A_1 at 20 seconds and A_2 for 2 hour 20 seconds are recorded at the wavelength of 500 nm. $\Delta A = A_2 - A_1$.

III. Calculation.

1. Tissue

A. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of oxidized o-anisome every milligram of tissue protein per minute.

$$\text{GOD(U/mg prot)} = \Delta A \div d \div \epsilon \times V_{\text{RT}} \div (\text{Cpr} \times V_{\text{S}}) \div T = 1333 \times \Delta A \div \text{Cpr}$$

B. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of oxidized o-anisome every gram of tissue per minute.

$$\text{GOD(U/g)} = \Delta A \div d \div \epsilon \times V_{\text{RT}} \div (W \div V_{\text{E}} \times V_{\text{S}}) \div T = 1333 \times \Delta A \div W$$

C. Bacteria or cells:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of oxidized o-anisome every 10^4 of bacteria or cells per minute.

$$\text{GOD(U/mL)} = \Delta A \div (\epsilon \times d) \times V_{\text{RT}} \div V_{\text{S}} \div T = 2.666 \times \Delta A$$

D. Serum:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of oxidized o-anisome every milliliter of serum per minute.

$$\text{GOD(U/mL)} = \Delta A \div d \div \epsilon \times V_{\text{RT}} \div V_{\text{S}} \div T = 1333 \times \Delta A$$

V_{RT} : Reaction total volume, 1.03 mL;

V_{S} : Sample volume, 0.1 mL;

V_{E} : Extract solution volume, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

D: Light path, 1 cm;

ϵ : The coefficient light extinction of oxidized of o-anisome, $7.5 \times 10^{-3} \text{ mL}/\mu\text{mol}/\text{cm}$;

T: Reaction time, 1 minute.

Note:

1. The activity of GOD is different in different homogenate tissues. Make 1-2 pre-experiment before testing. If $A_2 - A_1 > 0.8$, the tissue activity is too high, so the Extract solution must be diluted into appropriate concentration to make $A_2 - A_1 < 0.8$, so as to improve the detection sensitivity. If $A_1 > A_2$ occurs during the experiment, the sample should be diluted to an appropriate concentration with the extract solution.

2. It is better for two people to do experiments at the same time. One person is colorimetric and the other is timing to ensure the accuracy of the experimental results.

Recent Product Citations:

[1] Yan Ma, Zhe Chi, Yanfeng Li, et al. Cloning, deletion, and overexpression of a glucose oxidase gene in *Aureobasidium* sp. P6 for Ca^{2+} -gluconic acid overproduction. *Annals of Microbiology*. October 2018; (IF1.431)

[2] Zhao S F, Jiang H, Chi Z, et al. Genome sequencing of *Aureobasidium pullulans* P25 and overexpression of a glucose oxidase gene for hyper-production of Ca^{2+} -gluconic acid[J]. *Antonie Van Leeuwenhoek*, 2019, 112(5): 669-678.

References:

[1] Han K, Wu Z, Lee J, et al. Activity of glucose oxidase entrapped in mesoporous gels[J]. *Biochemical engineering journal*, 2005, 22(2): 161-166.

Related Products:

NA0380/NA0612 Hydrogen Peroxide(H_2O_2) Content Assay Kit
NA0789/NA0548 Xanthine Oxidase(XOD) Activity Assay Kit
NA0771/NA0530 Diamine Oxidase(DAO) Activity Assay Kit
NA0772/NA0531 Protein Carbonyl Content Assay Kit