SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Glucose Oxidase (GOD) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: AK0521

Size: 100T/96S

Components:

Extract solution: Liquid 110 mL×1, store at 4℃ .

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

Reagent II: Liquid 3 mL×1, store at 4℃ .

Reagent III: Liquid 1 mL×1, store at -20℃ . Store separately 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 H_2O_2。 GOD is one of the metabolic pathways of producing reactive oxygen in organisms.

When glucose is catalyzed to produce H_2O_2 by GOD. Then peroxidase catalyzes the production of H_2O_2 into oxygen, which oxidize o-dianisidine to form colored substance. The color depth is linear with glucose oxidase activity.

Required but not provided:

Spectrophotometer/Microplate reader, water-bath, centrifuge, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Protocol:

I. Sample Preparation.

1. Tissue: Homogenize 0. 1g of tissue sample in 1 mL of Extract solution in ice bath. Centrifuge at 8000×g for 10 minutes at 4℃ 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℃ for 10 minutes, the supernatant is taken and placed on ice for testing.

3.Serum: Detect directly.

II. Determination procedure.

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 500 nm, set zero with distilled water.

2. Working solution: Mix thoroughly 15 mL of Reagent I with 3 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	174
Reagent III	6
Mix thoroughly, incubate at 37℃ (mammals) or 25℃ (other animals) water bath for 5 minutes.	
Sample	20

Incubate working solution and Reagent III at 37℃ water bath before testing. Then the above reagents are added to the micro cuvette/96 well flat-bottom plate in order, and the timing began at the same time as the samples are added. Then the initial absorbance A1 at 20 seconds and A2 for 2 hour 20 seconds are recorded at the wavelength of 500 nm. ΔA=A2-A1.

III. Calculation.

A. Micro glass cuvette

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.

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

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.

$GOD(U/g)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(W\div V_E\times V_S)\div T=1333\times\Delta A\div W$

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.

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

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.

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

V_{RT}: Reaction total volume, 0.2 mL;

V_S: Sample volume, 0.02 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, 7.5×10^{-3} mL/μmol/cm;

T: Reaction time, 1 minute.

B. 96 well plate

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.

$GOD(U/mg\ prot)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(cpr\times V_S)\div T=2222\times\Delta A\div Cpr$

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.

$GOD(U/g)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(W\div V_E\times V_S)\div T=2222\times\Delta A\div W$

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.

$GOD(U/mL)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div V_S\div T=4.443\times\Delta A$

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.

$GOD(U/mL)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div V_S\div T=2222\times\Delta A$

V_{RT}: Reaction total volume, 0.2 mL;

V_S: Sample volume, 0.02 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

d: Light path, 0.6 cm;

ε: The coefficient light extinction of oxidized of o-anisome, 7.5×10^{-3}mL/μmol/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 A2-A1>0.8, the tissue activity is too high, so the Extract solution must be diluted into appropriate concentration to make A2-A1<0.8, so as to improve the detection sensitivity. If A1 > A2 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:

AK0362/AK0361	Hydrogen Peroxide(H2O2) Content Assay Kit
AK0490/AK0489	Xanthine Oxidase(XOD) Activity Assay Kit
AK0458/AK0457	Diamine Oxidase(DAO) Activity Assay Kit
AK0460/AK0459	Protein Carbonyl Content Assay Kit