Glucose Oxidase (GOD) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC0695

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent Name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I	Liquid 15 mL×1	2-8℃
Reagent II	Liquid 3 mL×1	2-8℃
Reagent III	Liquid 1 mL×1	-20℃

Solution Preparation:

1. **Preparation of working liquid:** Take 15 mL of Reagent I and 3 mL of Reagent II and mix well (prepare when the solution will be used).
2. **Reagent III:** Store separately after melting, it can be stored at -20℃.

Product 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:

Visible spectrophotometer/Microplate reader, water-bath, desk centrifuge, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Operation procedure:

I. Sample Preparation.(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

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. 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. Calculate by sample 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.

$GOD(U/mg\ prot)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(Cpr\times V_S)\div T=0.667\times\Delta A\div Cpr$

2. Calculate by sample mass

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.

$GOD(U/g)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(W\div V_E\times V_S)\div T=0.667\times\Delta A\div W$

3. Calculate by the number of 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.

$GOD(U/10^4\ cell)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(500\div V_E\times V_S)\div T=1.333\times10^{-3}\times\Delta A\div W$

4. Calculate by liquid volume

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.

$GOD(U/mL)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div V_S\div T=0.667\times\Delta A$

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, 2 hours;

500: Number of bacteria or cells, 5 million.

B. 96 well plate

1. Calculate by sample 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.

$GOD(U/mg\ prot)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(Cpr\times V_S)\div T=1.111\times\Delta A\div Cpr$

2. Calculate by sample mass

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.

$GOD(U/g)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(W\div V_E\times V_S)\div T=1.111\times\Delta A\div W$

3. Calculate by the number of 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.

$GOD(U/mL)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(500\div V_E\times V_S)\div T=2.222\times10^{-3}\times\Delta A\div W$

4. Calculate by liquid volume

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.

$GOD(U/mL)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div V_S\div T=1.111\times\Delta A$

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, 2 hours;

500: Number of bacteria or cells, 5 million.

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] Lv C, Yang X, Wang Z, Ying M, Han Q, Li S. Enhanced Performance of Bioelectrodes Made with Amination-Modified Glucose Oxidase Immobilized on Carboxyl-Functionalized Ordered Mesoporous Carbon. Nanomaterials (Basel). 2021 Nov 16;11(11):3086. doi: 10.3390/nano11113086. PMID: 34835850; PMCID: PMC8617758.

[2] Pan Y, Zhu Y, Xu C, Pan C, Shi Y, Zou J, Li Y, Hu X, Zhou B, Zhao C, Gao Q, Zhang J, Wu A, Chen X, Li J. Biomimetic Yolk-Shell Nanocatalysts for Activatable Dual-Modal-Image-Guided Triple-Augmented Chemodynamic Therapy of Cancer. ACS Nano. 2022 Nov 22;16(11):19038-19052. doi: 10.1021/acsnano.2c08077. Epub 2022 Oct 31. PMID: 36315056.

[3] Li Y, Su L, Zhang Y, Liu Y, Huang F, Ren Y, An Y, Shi L, van der Mei HC, Busscher HJ. A Guanosine-Quadruplex Hydrogel as Cascade Reaction Container Consuming Endogenous Glucose for Infected Wound Treatment-A Study in Diabetic Mice. Adv Sci (Weinh). 2022 Mar;9(7):e2103485. doi: 10.1002/advs.202103485. Epub 2022 Jan 22. PMID: 35064773; PMCID: PMC8895150.

[4] Yu Y, Lin R, Yu H, Liu M, Xing E, Wang W, Zhang F, Zhao D, Li X. Versatile synthesis of metal-compound based mesoporous Janus nanoparticles. Nat Commun. 2023 Jul 17;14(1):4249. doi: 10.1038/s41467-023-40017-2. PMID: 37460612; PMCID: PMC10352278.

[5] Chen J, Wang X, Yuan Y, Chen H, Zhang L, Xiao H, Chen J, Zhao Y, Chang J, Guo W, Liang XJ. Exploiting the acquired vulnerability of cisplatin-resistant tumors with a hypoxia-amplifying DNA repair-inhibiting (HYDRI) nanomedicine. Sci Adv. 2021 Mar 26;7(13):eabc5267. doi: 10.1126/sciadv.abc5267. PMID: 33771859; PMCID: PMC7997498.

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:

BC3590/BC3595	Hydrogen Peroxide(H2O2) Content Assay Kit
BC1090/BC1095	Xanthine Oxidase(XOD) Activity Assay Kit
BC1280/BC1285	Diamine Oxidase(DAO) Activity Assay Kit
BC1270/BC1275	Protein Carbonyl Content Assay Kit