Glucose Dehydrogenase (GCDH) Activity Assay Kit

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

Detection instrument: Spectrophotometer/Microplate reader

Cat No: BC2695

Size: 100T/96S

Components:

Extract solution: Liquid 100 mL×1.Store at 2-8℃.

Reagent I: Liquid 25 mL×1. Store at 2-8℃.

Reagent II: Powder×1. Store at 2-8℃. Add 7.5 mL of Reagent I to dissolve it before use. It could be stored at 2-8℃ for eight weeks.

Reagent III: Powder×1. Store at -20℃ and protected from light. Add 5 mL of Reagent I to dissolve it before use. It could be stored at -20℃ for four weeks after dispensing to avoid repeated freezing and thawing.

Working solution: Reagent I, Reagent II and Reagent III are mixed by the ratio of 4:3:2 to make working solution according to sample number. Mix thoroughly. Keep it at 37℃ for 10 min before use

Product Description:

GCDH (EC 1.1.1.47) catalyzes D-glucose and NAD(P) to form D-Gluconic acid and NAD(P)H, which are mainly found in the liver of many microorganisms and higher animals. GCDH is an ideal enzyme for the preparation of high content fructooligosaccharide, and it is also a diagnostic enzyme for clinical blood glucose determination, which can be widely used in food industry and pharmaceutical industry.

GCDH catalyzes the formation of d-Gluconic acid and NADH from D-glucose and NAD. The activity of glucose dehydrogenase can be reflected by the change of absorbance value of NADH at 340 nm.

Required material

Spectrophotometer/microplate reader, tabletop centrifuge, water bath, micro quartz cuvette/96 well flat-bottom UV plate, transferpettor, mortar/homogenizer, ice and distilled water.

Procedure:

I. Extraction of crude enzyme solution:

1. **Tissue sample:** The proportion of tissue mass (g): extraction solution volume (mL)=1:5~10 (it is recommended to weigh about 0.1g of tissue, add 1 mL of extraction solution), carry out homogenization in the ice bath. Centrifuge at 8000×g for 10 minutes at 4℃, take the supernatant and put it on ice for testing.
2. **Bacteria/cells:** Collect the cells/bacteria into the centrifuge tube, and then discard the supernatant after centrifugate. The proportion of the number of cells (10^4): the volume of the extraction solution (mL) is 500~1000:1 (it is recommended to add 1 mL of the extraction solution to 5 million cells/bacteria), break the cells/bacteria in the ice bath by ultrasonic (power 200W, ultrasonic 3s, interval 7s, total time 5 minutes). Then centrifuge at 8000 ×g for 10 minutes at 4℃, take the supernatant and put it on ice for testing.

3. **Serum (plasma):** Direct detection.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader 30 minutes, adjust wavelength to 340 nm, set spectrophotometer zero with distilled water.
2. Operation table: add the following reagents into the micro quartz cuvette/96 well UV plate respectively:

Reagent (μL)	Blank tube (B)	Test tube (T)
Working solution	180	180
Distilled water	20	-
Sample	-	20
Add the working solution and start timing, mix it immediately, measure the absorbance value A1 at 340 nm for 10s, quickly put it in a 37℃ water bath or incubator for 1 minutes (the temperature can be adjusted to 37℃ if the microplate reader has temperature control function), take out the absorbance value A2 at 70s, calculate the $\Delta A_T=A2_T-A1_T$, $\Delta A_B=A2_B-A1_B$, $\Delta A=\Delta A_T-\Delta A_B$. Blank tube only needs to be done 1-2 times.		

III. GCDH enzyme activity calculation:

1. Calculate according to micro quartz cuvette:

1) Calculated by protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1 nmol of NADH per minute every per milligram of protein.

The activity of GCDH(U/mg prot)＝$\Delta A\div(\varepsilon\times d)\times10^9\times V_{RT}\div(V_S\times Cpr)\div T=1607.7\times\Delta A\div Cpr$

2) Calculated by sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1 nmol of NADH per minute every gram of sample.

The activity of GCDH(U/g weight)＝$\Delta A\div(\varepsilon\times d)\times10^9\times V_{RT}\div(V_S\times W\div V_{ST})\div T=1607.7\times\Delta A\div W$

3) Calculated by bacteria or cell amount:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1 nmol of NADH per minute every 10^4 bacteria/cells.

The activity of GCDH(U/10^4 cell)＝$\Delta A\div(\varepsilon\times d)\times10^9\times V_{RT}\div(V_S\div V_{ST}\times N)\div T=1607.7\times\Delta A\div N$

4) Calculated by liquid volume

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1 nmol of NADH per minute every milliliter sample.

The activity of GCDH(U/mL)＝$\Delta A\div(\varepsilon\times d)\times10^9\times V_{RV}\div V_S\div T=1607.7\times\Delta A$

ε: Molar extinction coefficient of NADH, 6.22×10^3 L/mol/cm;

d: Optical diameter of cuvette, 1 cm;

10^9: Unit conversion coefficient, 1 mol = 10^9 nmol;

V_{RV}: The total volume of the reaction system, 2×10^{-4} L;

V_S: The volume of the sample in the reaction system, 0.02 mL;

V_{ST}: The volume of the added extract, 1 mL;

Cpr: Supernatant protein concentration, mg/mL;

W: Sample weight, g;

T: React time, 1 minute;

N: Amount of Bacteria/cell, 10^4 cells as unit;

2. Calculate according to 96 well UV plate:

Change the d-1 cm in the above formula to d-0.6 cm (the optical diameter of cuvette) for calculation.

Note:

1. Put the supernatant of sample extraction on ice to be tested, and it is recommended to complete the test within the same day after sample extraction.
2. When A1 or A2 is greater than 1.7, it is recommended to dilute the sample with extraction solution before determination.
3. When ΔA is greater than 1.5, it is recommended to dilute the sample with extraction solution before determination.

Experimental example:

1. Take 0.1g liver and add 1 mL of Extract solution for homogenate. After taking the supernatant, operate according to the determination steps. Measure with micro quartz cuvette and calculate $\Delta A_T = A2_T - A1_T = 0.5806-0.5291 = 0.0515$, $\Delta A_B = A2_B-A1_B = 0.0294-0.0294 = 0$, $\Delta A=\Delta A_T-\Delta A_B =0.0515-0 = 0.0515$.

   GCDH activity (U/g weight) = $1607.7\times\Delta A \div W = 1607.7\times0.0515 \div 0.1 = 827.966$ U/g weight.

Related Products:

BC2590/BC2595	Neutral Xylanase Activity Assay Kit
BC2620/BC2625	β-xylosidase Activity Assay Kit
BC2710/BC2715	Total Carbohydrate Content Assay Kit
BC3330/BC3335	Glycogen synthase(GCS) Activity Assay Kit