

## Glucose Dehydrogenase (GCDH) Activity Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Cat No:** BC2690

**Size:** 50T/48S

### Components:

**Extract solution:** Liquid 60 mL×1. Store at 2-8°C.

**Reagent I:** Liquid 50 mL×1. Store at 2-8°C.

**Reagent II:** Powder×1. Store at 2-8°C. Add 15 mL of Reagent I to dissolve it before use. It could be stored at 2-8°C for eight weeks.

**Reagent III:** Powder×2. Store at -20°C and protect from light. Add 5 mL of Reagent I into each bottle to dissolve it before use. It could be stored at -20°C 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°C 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, centrifuge, water bath, 1 mL quartz cuvette, transferpeltor, mortar/homogenizer, ice and distilled water.

### Procedure:

#### I. Extraction of crude enzyme solution:

- 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°C, take the supernatant and put it on ice for testing.
- 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<sup>4</sup>): 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°C, take the supernatant and put it on ice for testing.

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

## II. Determination:

1. Preheat the spectrophotometer 30 minutes, adjust wavelength to 340 nm, set zero with distilled water.

2. Operation table: add the following reagents into the 1 mL quartz cuvette respectively:

Reagent (μL)	Blank tube (B)	Test tube (T)
Working solution	900	900
Distilled water	100	-
Sample	-	100

Add the working solution and start timing, mix it immediately, measure the absorbance value A1 at 340 nm for 10s, quickly put it in 37°C-water bath or incubator for 1 minutes. Take it out and dry it quickly, measured the absorbance value A2 at 1min10s, calculate the  $\Delta A_T = A_{2T} - A_{1T}$ ,  $\Delta A_B = A_{2B} - A_{1B}$ ,  $\Delta A = \Delta A_T - \Delta A_B$ . Blank tube only needs to be done 1-2 times.

## III. GCDH activity calculation:

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.

$$\text{GCDH activity (U/mg prot)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{RT} \div (V_S \times C_{Pr}) \div T = 1607.7 \times \Delta A \div C_{Pr}$$

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.

$$\text{GCDH activity (U/g weight)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{RT} \div (V_S \times W \div V_{ST}) \div T = 1607.7 \times \Delta A \div W$$

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<sup>4</sup> bacteria/cells.

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

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.

$$\text{GCDH activity (U/mL)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{RV} \div V_S \div T = 1607.7 \times \Delta A$$

$\epsilon$ : Molar extinction coefficient of NADH, 6.22×10<sup>3</sup> L/mol/cm;

d: Optical diameter of cuvette, 1 cm;

10<sup>9</sup>: Unit conversion coefficient, 1 mol = 10<sup>9</sup> nmol;

V<sub>RV</sub>: The total volume of the reaction system, 1×10<sup>-3</sup> L;

$V_s$ : The volume of the sample in the reaction system, 0.1 mL;

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

$C_{pr}$ : Supernatant protein concentration, mg/mL;

$W$ : Sample weight, g;

$T$ : React time, 1 minute;

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

#### 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  $A_1$  or  $A_2$  is greater than 1, it is recommended to dilute the sample with extraction solution before determination.
3. When  $\Delta A$  is greater than 1, 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  $\Delta A_T = A_{2T} - A_{1T} = 0.726 - 0.682 = 0.044$ ,  $\Delta A_B = A_{2B} - A_{1B} = 0.031 - 0.031 = 0$ ,  $\Delta A = \Delta A_T - \Delta A_B = 0.044 - 0 = 0.044$ . Calculate the enzyme activity according to the sample weight:  
 $GCDH \text{ activity (U/g weight)} = 1607.7 \times \Delta A \div W = 1607.7 \times 0.044 \div 0.1 = 707.388 \text{ U/g weight}$ .

#### Related Products:

- |               |  |
|---------------|--|
| BC2590/BC2595 | Neutral Xylanase Activity Assay Kit    |
| BC2620/BC2625 | $\beta$ -xylosidase Activity Assay Kit |