Beta-glucosidase Assay Kit

Cat #: orb1500022 (manual)

Visible Spectrophotometer

Size: 50T /24S

Product composition and storage conditions :

No.	Specifications	Storage conditions
Extraction solution - ES02	50mL×1 bottle	Store at 4°C
orb1500022-A	Powder×2 bottle	Store at -20°C Before use, add 10mL of distilled water into each bottle, dissolve it fully; the remaining reagent can be stored at -20°C for 4 weeks, avoid repeated freezing and thawing.
orb1500022-B	25mL×1 bottle	Store at 4℃
orb1500022-C	80mL×1 bottle	Store at 4℃
orb1500022-Standard (5 µmol/mL)	1mL×1 tube	Store at 4℃

※Before the formal measurement, be sure to take 2-3 samples with large expected differences for predetermination.

Introduction

Significance: B-GC (EC 3.2.1.21) is widely found in animals, plants, microorganisms and cultured cells. It catalyzes the hydrolysis of β-glycosidic bonds, β-GC hydrolyzes terpene aroma precursors to make glycosides bond to free state, thus producing aroma. β-GC hydrolyzes cellulose disaccharides and cellulose oligosaccharides to produce glucose B-GC can hydrolyze cerasosides in plants and release HCN, which can prevent insects from feeding on them.

Principle: B-GC decomposes p-nitrobenzen-β-d-glucopyranoside to form p-nitrophenol, which has the largest absorption peak at 400 nm.

Own suppliers: Visible spectrophotometer,1 ml glass colorimeter, centrifuge, water bath, adjustable pipette, mortar, ice and distilled water.

Extraction of crude enzyme solution:

1. Bacteria or culture cells: collect bacteria or cells into the centrifuge tube, centrifuge and discard the supernatant; According to the number of bacteria or cells (10^4), extraction solution ES02 volume (mL) is 500 ~ 1000:1 ratio (5 million bacteria or cells are recommended to add 1 mL extraction ES02) , the bacteria or cells were broken by ultrasonic wave (ice bath, power 20% or 200 W, ultrasonic 3 s, interval 10 s, repeat 30 times) ; Then centrifuge for 10 min at 15000 g, 4°C, and take the supernatant and place it on ice for testing.

2. Tissue： According to the ratio of tissue mass (g) : ES02 volume (mL) : 1:5 ~ 10(about 0.1 g tissue should be taken and 1 mL ES02 should be added) , homogenized in ice bath, centrifuged at 15000 g 4C for 10 min, the supernatant was taken and put it on ice for testing.

3. Liquid sample: use and test directly.

Measurement operation:

1. Preheat the spectrophotometer for at least 30 minutes, adjust the wavelength to 400 nm, and set it to zero with the distilled water.

2. Dilution of standard solution: 5 μmol/mL standard solution was diluted to 300,200,100,50,25,10,0 nmol/mL standard solution with distilled water before use.

3. Sampling table:

Reagent name	Measuring tube (ul)	Control tube (ul)	Standard tube (ul)
orb1500022-A	400		
orb1500022-B	500	500	
Sample	100	100	
Quickly mix, put into 37°C water bath for 30min, then immediately put into 95°C water bath for 5min (cover tightly, to prevent water loss) , cooling with flow water and then mix fully (to ensure constant concentration)			
orb1500022-A		400	
Mix fully, then centrifuge at 8000g, 4°C for 5min, take supernatant			
Supernatant	500	500	
Standard			500
orb1500022-C	1000	1000	1000

Mix fully, stay at room temperature for 2min, the absorption value was determined at 400 nm, and record it as A-measuring, A-control, A-standard and A- blank respectively. Calculated ΔA-measuring = A-measure - A-control, ΔA-standard = A-standard - a-blank. A control tube is required for each measuring tube. Standard curve and blank tube only need to test 1-2 times.

β -GC activity calculations:

1. Standard curve establishment: according to the concentration of standard tube (x, nmol/mL) and absorbance (y, ΔA-standard) to make the standard curve. According to the standard curve, the sample concentration x (nmol/mL) was calculated by introducing ΔA (y, ΔA-measuring) into the formula.

2. Enzyme activity calculation:

(1) Calculated by protein concentration of sample:

Definition of unit: production of 1nmol p-nitrophenol per hour per mg of tissue protein is defined as a unit of enzyme activity.

β-GC activity (U/mg prot) = $(x \times V1) \div (V2 \times Cpr) \div T = 20x \div Cpr$

Protein concentration quantification is recommended using our BCA Protein Assay Kit (orb1085948).

(2) Calculated by sample fresh weight:

Definition of units: Production of 1nmol p-nitrophenol per hour per g of tissue is defined as a unit of enzyme activity.

β-GC activity (U/g mass) $= (x \times V1) \div (W \times V2 \div V3) \div T = 20x \div W$

(3) Calculated by number of bacteria or cells:

Definition of Units: Production of 1nmol p-nitrophenol per hour per 10000 bacteria or cells is defined as an enzyme activity unit.
Β -GC activity (U/10^4 cell)$= (x \times V1) \div (500 \times V2 \div V3) \div T = 0.02x$

Note: Cpr: protein concentration of sample, mg/mL, protein concentration needs to be determined separately; V1： Total volume of reaction, 1mL; V2: Sample volume added in reaction, 0.1mL; V3: Volume of extraction solution added, 1mL; W: Sample mass, g; 500: number of cells or bacteria, 5 million; T: Reaction time, 0.5h.

The extraction solution contains components that denature the protein, so the protein needs to be re-extracted for determination when calculated according to the protein concentration.