Beta-glucosidase Assay Kit

Cat #: orb1500021 (manual)

Trace method

Size: 100T / 48S

Product composition and storage conditions :

No.	Specifications	Storage conditions
Extraction solution -ES02	Liquid 100mL×1 bottle	Store at 4℃
orb1500021-A	Powder×1 bottle	Store at -20℃; Before use, add 12mL distilled water into each bottle immediately, dissolve it fully; the remaining reagent needs to be stored by -20 C.
orb1500021-B	Liquid 15mL×1 bottle	Store at 4℃
orb1500021-C	Liquid 15mL×1 bottle	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/plate reader, centrifuge, water bath, adjustable pipette, micro quartz cuvette/96-wells plate, 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 (104), 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℃, 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 visible spectrophotometer or plate reader for at least 30 minutes, adjust the wavelength to 400 nm, and set it to zero with the distilled water.

2. Sampling table:

Reagent name	Measuring tube (ul)	Control tube (ul)
orb1500021-A	120	
orb1500021-B	150	150
Sample	30	30
Quickly mix, put into 37℃ water bath for 30min, then immediately put into 95℃ water bath for 5min (cover tightly, to prevent water loss) , cooling with flow water and then mix fully (to ensure constant concentration)		

orb1500021-A		120
Mix fully, then centrifuge at 8000g, 4℃ for 5min, take supernatant		
Supernatant	70	70
orb1500021-C	130	130
Mix fully, stay at room temperature for 2min, the absorption value was determined at 400 nm, calculated ΔA-measuring = A-measure - A-control, a control tube is required for each measuring tube.		

β -GC activity calculations:：

a. The calculation formula for determination with microquartz cuvette is as follows

The regression equation determined under standard conditions is y = 0.32x -0.0027； x is the concentration of standard (nmol/mL), y is the absorbance value.

(1) Calculated by protein concentration of sample:

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

β-GC activity (nmol/h/mg prot) = [(ΔA+0.0027) ÷0.32×V1]÷(V2×Cpr)÷T = 62.5×(ΔA+0.0027)÷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 gram of tissue is defined as a unit of enzyme activity.

β-GC activity (nmol/h/g fresh weight) = [(ΔA+0.0027)÷0.32×V1]÷(W×V2÷V3)÷T = 62.5×(ΔA+0.0027) ÷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 (nmol/h/10^4 cell) = [(ΔA+0.0027)÷0.32×V1]÷(500×V2÷V3)÷T = 0.125×(ΔA+0.0027)

Note: Cpr：protein concentration of sample，mg/mL, protein concentration needs to be determined separately；V1：Total volume of reaction, 0.3mL；V2：Sample volume added in reaction, 0.03mL；V3：Volume of extraction solution added, 1mL；W：sample weight，g；500：number of cells or bacteria, 5 million; T: Reaction time, 0.5h.

b. The formula for determination with 96-well plates is as follows

The regression equation determined under standard conditions is y = 0.16x -0.0027；x is the concentration of standard (nmol/mL) ，y is the absorbance value.

(1) Calculated by protein concentration of sample:

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

β-GC activity (nmol/h/mg prot) = [(ΔA+0.0027) ÷0.16×V1]÷(V2×Cpr)÷T = 125×(ΔA+0.0027)÷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 tissues is defined as an enzyme activity unit.

β-GC activity (nmol/h/g fresh weight) = [(ΔA+0.0027)÷0.16×V1]÷(W×V2÷V3)÷T = 125×(ΔA+0.0027) ÷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 (nmol/h/104 cell) = [(ΔA+0.0027) ÷0.16×V1]÷(500×V2÷V3)÷T = 0.25×(ΔA +0.0027)

Note: Cpr：protein concentration of sample，mg/mL, protein concentration needs to be determined separately；V1：Total volume of reaction, 0.3mL；V2：Sample volume added in reaction, 0.03mL；V3：Volume of extraction solution added, 1mL；W：Sample weight， g；500：number of cells or bacteria, 5 million; T: Reaction time, 0.5h.