Starch branching enzyme(SBE)Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: BC1865

Size:100T/48S

Components:

Extract solution: Liquid 50 mL×1. Storage at 4℃.

Reagent I: Liquid 10 mL×1. Storage at 4℃.

Reagent II: Powder×2. Storage at 4℃. Add 0.6 mL of distilled water before use. Heat slowly and gradually till boiling to make it fully dissolved. It could be stored at 4℃ for four weeks.

Reagent III: Liquid 13 mL×1. Storage at 4℃.

Reagent IV: Liquid 2.5 mL×1. Storage at 4℃.

Product Description:

Starch branching enzyme(SBE) exists mainly in plants, is a key enzyme of amylopectin biosynthesis. The determination of SBE activity is of great significance in the study of starch biosynthesis, selection of high-quality crop varieties and genetic improvement of quality.

The complex formed by the combination of starch and iodine has a characteristic absorption peak at 660nm. SBE can cut off the side branches of amylopectin, thus reducing the absorption value of starch iodine complex at 660 nm. In a certain time, the percentage of absorbance decrease can reflect SBE activity.

Reagents and Equipments Required but Not Provided:

Spectrophotometer/microplate reader, water bath, desk centrifuge, adjustable transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Procedure:

I.Sample preparation:

Add 1 mL of Extract solution to 0.1 g of tissue. Homogenized on ice bath. Centrifuge at 15000 g for 15 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

II. Detection

1) Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 660 nm, and set spectrophotometer counter to zero with distilled water.

2) Add the following reagents in 1.5 mL EP tubes:

Reagent	Contrast tube (C)	Test tube (T)
Deactivated crude enzyme (μL)	63	-
Crude enzyme (μL)	-	63
Reagent I (μL)	80	80
Reagent II (μL)	8	8

Mix thoroughly and incubate at 37℃ for 20 minutes. then place the tubes in a boiling water bath for 1 minute(cover tightly to prevent moisture loss) and rapid cooling by ice bath.		
Reagent III (μL)	124	124
Reagent IV (μL)	25	25
Mix thoroughly and stand for 10 minutes. Take 200 μL of the supernatant to detect the absorbance at 660 nm, record as A_C and A_T respectively. Each test tube requires a contrast tube.		

Note: If there is turbidity in the sample, it is recommended to take the supernatant for determination after centrifugation.

III. Calculation:

1. Detected by micro glass cuvette

1) Tissue protein concentration

Unit definition: Enzyme activity is expressed as a percentage decrease in absorbance at a wavelength of 660 nm. One unit of enzyme activity is defined as the amount of enzyme reduces 1% of iodine blue value in the reaction system per minute every mg protein.

SBE Activity(U/mg prot)=$(A_C-A_T)\div A_C\times 100\%\div 1\%\div(Cpr\times Vs)\times Vrv\div T=(A_C-A_T)\div A_C\div Cpr\times 23.8$

2) Tissue weight

Unit definition: Enzyme activity is expressed as a percentage decrease in absorbance at a wavelength of 660 nm. One unit of enzyme activity is defined as the amount of enzyme reduces 1% of iodine blue value in the reaction system per minute every g sample.

SBE Activity(U/g weight)=$(A_C-A_T)\div A_C\times 100\%\div 1\%\div(W\times Vs\div Ve)\times Vrv\div T=(A_C-A_T)\div A_C\div W\times 23.8$

Vs: Sample volume (mL), 0.063 mL;

Ve: Extract solution volume, 1 mL;

Cpr: Supernatant sample protein concentration, mg/mL;

Vrv: Total reaction volume, 0.3 mL;

T: Reaction time, 20 minutes;

W: Sample weight, g.

2. Detected by 96 well flat-bottom plate

1) Tissue protein concentration

Unit definition: Enzyme activity is expressed as a percentage decrease in absorbance at a wavelength of 660 nm. One unit of enzyme activity is defined as the amount of enzyme reduces 0.5% of iodine blue value in the reaction system per minute every mg protein.

SBE Activity(U/mg prot)=$(A_C-A_T)\div A_C\times 100\%\div 0.5\%\div(Cpr\times Vs)\times Vrv\div T=(A_C-A_T)\div A_C\div Cpr\times 47.6$

2) Tissue weight

Unit definition: Enzyme activity is expressed as a percentage decrease in absorbance at a wavelength of 660 nm. One unit of enzyme activity is defined as the amount of enzyme reduces 0.5% of iodine blue

value in the reaction system per minute every g sample.

SBE Activity(U/g weight)=(A_C-A_T)÷A_C×100%÷0.5%÷(W×Vs÷Ve)×Vrv÷T=(A_C-A_T)÷A_C÷W×47.6

Vs: Sample volume (mL), 0.063 mL;
Ve: Extract solution volume, 1 mL;
Cpr: Supernatant sample protein concentration, mg/mL;
Vrv: Total reaction volume, 0.3 mL;
T: Reaction time, 20 minutes;
W: Sample weight, g.

Note:

1. The crude enzyme solution of different samples can be added into different care tubes, and then concentrated for 1min boiling water bath treatment.
2. If there is a precipitate in Reagent I, it should be fully dissolved and mixed before adding.

Experimental example:

1. Take 0.1g peach leaves to 1ml extract solution, grinding on ice and 15000g, 4℃centrifuge for 15min, operate as the procedure after taking the supernatant, A_T=0.277, A_C=0.399, calculate enzyme activity by sample weight: SBE activity（U/g weight)= (A_C-A_T)÷A_C÷W×23.8=72.77 U/g weight.

Recent Product citations：

[1] Peitong Wang Xi Chen Xuan Xu,et al. Arsenate induced chlorosis 1/ translocon at the outer envolope membrane of chlooplasts 132 protects chloroplasts from Arsenic Toxicity. Plant physiology. October 2018;(IF6.305)

References：

[1] Jiang H, Dian W, Wu P. Effect of high temperature on fine structure of amylopectin in rice endosperm by reducing the activity of the starch branching enzyme[J]. Phytochemistry, 2003, 63(1): 53-59.

Related products：

BC3290/BC3295 Bound Station amylosynthease Activity Assay Kit
BC1850/BC1855 Soluble Starch Synthase(SSS) Activity Assay Kit
BC4270/BC4275 Amylopectin Content Assay Kit
BC4260/BC4265 Amylose Content Assay Kit

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