Amylopectin Content Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat No: BC4275

Size: 100T/96S

Components:

Reagent I: Liquid 110 mL×1. Store at 4℃.

Reagent II: Ether 100 mL×1. Required but not provided. Store at 4℃.

Reagent III: Liquid 55 mL×1. Store at 4℃.

Reagent IV: Reagent III and distilled water are mixed by the ratio of 9 mL:91 mL to make Reagent IV. Prepare when the solution will be used. It could be stored at 4℃ for six months.

Reagent V: Liquid 2 mL×1. Store at 4℃.

Reagent VI: Liquid 10 mL×1. Store at 4℃.

Standard: Powder×1. Store at 4℃. 10 mg amylopectin. Add 0.1 mL of absolute ethanol and 0.9 mL of Reagent III before use. Covering the lid and sealing, then boiling until it fully dissolved to produce a 10 mg/mL amylopectin standard solution. It could be stored at 4℃ for six months.

Product Description:

Amylopectin generally consists of thousands of glucose residues. The processing, physicochemical properties, gelatinization temperature, and other aspects of starch products are directly affected by the ratio and content of amylose and amylopectin in starch.

Amylopectin reacts with iodine to form a red purple complex, which results in a colorimetric product proportional to the amount of amylopectin. Using ethanol to separate the soluble sugar and starch in the sample, then the content of amylopectin can be obtained by reacting with iodine.

Reagents and Equipment Required but Not Provided:

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

Procedure:

I. Sample preparation:

Weigh 0.005 g of dried sample and grind it in a mortar, add 1 mL of Reagent I and transfer to one EP tube after homogenizing. Incubate at 80℃ for 30 minutes. Cool to room temperature in an ice bath. Centrifuge at 3000×g for 5 minutes at 25℃, discard the supernatant and leave the sediment. Add 1 mL of Reagent II (ether) to the sediment and shake for 5 minutes. Centrifuge at 3000×g for 5 minutes at 25℃, discard the supernatant and leave the sediment. Dissolve the sediment with 5 mL of Reagent IV, and incubate at 90℃ for 10 minutes. Cool to room temperature in an ice bath. Centrifuge at 3000×g for 5 minutes at 25℃ to remove insoluble materials and take the supernatant for testing.

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II. Detection

1) Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 530 nm and 755 nm, set spectrophotometer counter to zero with distilled water.

2) Standard working solution: dilute the 10 mg/mL standard solution to 1, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05 mg/mL with Reagent IV.

3) Add the following reagents in 1.5 mL EP tubes or 96 well plates:

Reagent	Test tube (T)	Standard tube (S)	Blank Tube (B)
Sample (μL)	40	-	-
Standard solution (μL)	-	40	-
Distilled water (μL)	-	-	40
Reagent V (μL)	15	15	15
Reagent VI (μL)	10	10	10
Distilled water (μL)	135	135	135
Mix thoroughly, take the supernatant to detect the absorbance at 530 nm and 755 nm. Under the 530nm, record as A_T, A_S and A_B respectively. Under the 755 nm, record as A'_T, A'_S and A'_B respectively. $\Delta A_T=(A_T-A_B)-(A'_T-A'_B)$, $\Delta A_S=(A_S-A_B)-(A'_S-A'_B)$. Standard curve and blank tube only need to be measured once or twice.			

II. Calculation:

1. Standard curve

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation y = kx + b, and bring ΔA_T into the equation to get x (mg/mL).

2. Calculation

Amylopectin Content (mg/g weight) = x ×Ve÷W=5x÷W

Ve: Reagent IV volume, 5 mL;

W: Sample weight, g.

Note:

1. It is recommended to complete the detection within 20 minutes after reaction to prevent the fading.

2. If the measured absorbance value exceeds the absorbance value in the linear range, you can increase the sample volume or dilute the sample before performing the measurement.

Experimental examples:

1. Take 0.01g red beans for sample processing. Dilute the supernatant 3 times and follow the measurement procedure.After determination with 96 well flat-bottom plate, calculate $\Delta A_T=(A_T-A_B)-(A'_T-A'_B)$=（0.351-0.059）-（0.190-0.048）=0.150. Bring the result into the standard curve y=0.5157x+0.0089, and calculate x=0.2736. The content is calculated according to the sample mass.

Amylopectin Content (mg/g weight) =5x÷W×3(dilution times)=410.4 mg/g weight.

Related products:

BC2040/BC2045 β-amylase Activity Assay Kit
BC0430/BC0435 ADPG Pyrophosphorylase(AGP) Activity Assay Kit
BC4255/BC4255 Starch Debranching Enzyme (DBE) Activity Assay Kit
BC4260/BC4265 Amylose Content Assay Kit

Technical Specifications:

Minimum Detection Limit：0.0193 mg/mL
Linear Range：0.025-1 mg/mL

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