Amylopectin Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat No: NA0305

Size:100T/96S

Components:

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

Reagent II: Ether 100 mL×1. Required but not provided.

Reagent III: Liquid 55 mL×1. Storage 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. Storage at 4℃ and use within 6 months of reconstitution.

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

Powder I: Powder×1. Storage at 4℃.

Powder II: Powder×1. Storage at 4℃.

Reagent VI: Mix Powder I and Powder II, dissolve the compound in distilled water to make up to 10mL. Storage at 4℃ and protect from light for one month.

Standard: Powder × 1 (10 mg amylopectin). Storage at 4℃ and protect from light. 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. Take 0.1 mL of 10 mg/mL amylopectin standard solution and add 0.9 mL of distilled water to prepare 1 mg/mL standard solution for use.

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.

II. Detection

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

2) Standard: Dilute the 1 mg/mL standard solution to 1, 0.8, 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. $\Delta A_T=(A_T-A_B)-(A'_T-A'_B)$, $\Delta A_S=(A_S-A_B)-(A'_S-A'_B)$.			

II. Calculation:

1. Standard curve

The concentration of standard solution as x-axis, ΔA_S as y-axis, obtain the equation y=kx+b. Take ΔA_T to the equation to acquire x value.

2. Calculation

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

Related products:

NA0723/NA0481 β-amylase Activity Assay Kit

NA0833/NA0591 ADPG Pyrophosphorylase(AGP) Activity Assay Kit

NA0321/NA0321 Starch Debranching Enzyme (DBE) Activity Assay Kit

NA0314/NA0313 Amylose Content Assay Kit

Technical Specifications:

Minimum Detection Limit：0.0193 mg/mL

Linear Range：0.025-1 mg/mL