Amylopectin Content Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: NA0306

Size:50T/48S

Components:

Reagent I: Liquid 60 mL×1. Storage at 4℃.

Reagent II: Ether 50 mL×1. Required but not provided.

Reagent III: Liquid 30 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 6 mL×1. Storage at 4℃.

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

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, water bath, desk centrifuge, adjustable transferpettor, 1 mL glass cuvette, mortar/ homogenizer, ether, absolute ethanol, 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 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 0.5, 0.4, 0.2, 0.1, 0.05, 0.025, 0.0125 mg/mL with Reagent IV.

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

Reagent	Test tube (T)	Standard tube (S)	Blank Tube (B)
Sample (μL)	200	-	-
standard solution (μL)	-	200	-
Distilled water (μL)	-	-	200
Reagent V (μL)	75	75	75
Reagent VI (μL)	50	50	50
Distilled water (μL)	675	675	675
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. $\Delta A_T=(A_T-A_B)-(A'_T-A'_B)$, $\Delta A_S=(A_S-A_B)-(A'_S-A'_B)$.			

III.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.Calculate $\Delta A_T=(A_T-A_B)-(A'_T-A'_B)=$（0.370-0.045）-（0.233-0.015）=0.107. Bring the result into the standard curve y=0.7564x-0.0006, and calculate x=0.1423.The content is calculated according to the sample mass.

Amylopectin Content (mg/g sample) =5x÷W×3(dilution times)=213.45mg/g sample.

Related products:

NA0723/NA0481 β-amylase Activity Assay Kit

NA0833/NA0591 ADPG Pyrophosphorylase(AGP) Activity Assay Kit

NA0322/NA0321 Starch Debranching Enzyme (DBE) Activity Assay Kit

NA0314/NA0313 Amylose Content Assay Kit

Technical Specifications:

Minimum Detection Limit：0.0044 mg/mL

Linear Range：0.0125-0.5 mg/mL