Amylose Content Assay Kit

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

Operation Equipment: Spectrophotometer /Microplate Reader

Cat No: NA0313

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 0.5 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 amylose). 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 amylose standard solution. Take 0.1 mL of 10 mg/mL amylose standard solution and add 0.9 mL of distilled water to prepare 1 mg/mL standard solution for use.

Product Description:

Amylose is a polysaccharide chain linked by d-glucosyl -(1,4) glycosidic bonds, which affects the edible quality and appearance quality of food, and is closely related to food safety.

Amylose reacts with iodine to form a blue complex, which results in a colorimetric product proportional to the amount of amylose. Using ethanol to separate the soluble sugar and starch in the sample, then the content of amylose 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 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. Determination

1) Preheat microplate reader or spectrophotometer for 30 minutes, adjust the wavelength to 550 nm and 485 nm, set zero with distilled water.

2) Dilute the 1 mg/mL standard solution to 0.4 mg/mL standard solution with Reagent IV.

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

Reagent	Test tube (T)	Standard tube (S)	Blank Tube (B)
Sample (μL)	20	-	-
0.4 mg/mL standard solution (μL)	-	20	-
Distilled water (μL)	-	-	20
Reagent V (μL)	4	4	4
Reagent VI (μL)	4	4	4
Distilled water (μL)	172	172	172
Mix thoroughly, take the supernatant to detect the absorbance at 550 nm and 485 nm. Under the 550 nm, record as A_T, A_S and A_B respectively. Under the 485 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:

Amylose Content (mg/g sample) $=\Delta A_T \div (\Delta A_S \div C_S) \times Ve \div W = 2\times \Delta A_T \div \Delta A_S \div W$

Cs: Standard concentration, 0.4 mg/mL;

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 A>0.95, the sample can be determined after being appropriately diluted with Reagent IV. If A<0.07, the volume of Reagent IV can be reduced during extraction.

Experimental examples:

1. Weigh 0.01 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. After determination

with 96 well plate, calculate $\Delta A_T=(A_T-A_B)-(A'_T-A'_B)$=（0.664-0.003）-（0.474-0.034）=0.221，$\Delta A_S=(A_S-A_B)-(A'_S-A'_B)$=（0.723-0.003）-（0.447-0.034）=0.307.The content is calculated according to the sample mass.

Amylose Content (mg/g sample) =$2\times\Delta A_T \div \Delta A_S \div W$=143.97mg/g sample.

Related products:

NA0813/NA0571 Starch Content Assay Kit

NA0820/NA0578 α-amylase Activity Assay Kit

NA0306/NA0305 Amylopectin Content Assay Kit

NA0676/NA0434 α-1,4-Glucan Glucohydrolace Activity Assay Kit

Technical Specifications:

Minimum Detection Limit：0.0019 mg/mL

Linear Range：0.025-0.8 mg/mL