Amylose Content Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC4260

Size:50T/48S

Components:

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

Reagent II: Ether 50 mL×1. Required but not provided. Store at 4℃.

Reagent III: Liquid 30 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. Storage at 4℃ and use within 6 months of reconstitution.

Reagent V: Liquid 1.5 mL×1. Store at 4℃.

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

Standard: Powder×1. Store at 4℃. 10 mg amylose. 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. It could be stored at 4℃ for six months

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, water bath, desk centrifuge, adjustable transferpettor, 1 mL glass cuvette, mortar/homogenizer, ether, absolute ethanol, ice and distilled water.

Procedure:

I. **Sample preparation:**

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.

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II. Determination

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

2) Standard working solution: dilute the standard solution of 10 mg/mL with Reagent IV to 0.4 mg/mL.

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

Reagent	Test tube (T)	Standard tube (S)	Blank Tube (B)
Sample (μL)	100	-	-
0.4 mg/mL standard solution (μL)	-	100	-
Distilled water (μL)	-	-	100
Reagent V (μL)	20	20	20
Reagent VI (μL)	20	20	20
Distilled water (μL)	860	860	860
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. $\Delta A_T=(A_T-A_B)-(A'_T-A'_B)$, $\Delta A_S=(A_S-A_B)-(A'_S-A'_B)$. Standard tube and blank tube only need to be measured once or twice.			

II. Calculation:

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

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>1, the sample can be determined after being appropriately diluted with Reagent IV. If A<0.03, 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. 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 weight) =$2\times\Delta A_T\div\Delta A_S\div W$=143.97mg/g weight.

Related products:

BC0700/BC0705 Starch Content Assay Kit
BC0610/BC0615 α-amylase Activity Assay Kit
BC4270/BC4275 Amylopectin Content Assay Kit
BC2670/BC2675 α-1,4-Glucan Glucohydrolace Activity Assay Kit

Technical Specifications:

Minimum Detection Limit：0.006 mg/mL
Linear Range：0.0125-0.6 mg/mL

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