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Ver.250301

Amylose/Amylopectin/Total Starch Content Assay Kit

BC6100(50Tests/24Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description:

Starch is composed of amylose and amylopectin. Amylose is a polysaccharide consisting of α -D-glucose units that are linked together by α -(1, 4) glycosidic bonds. The glucose units in amylopectin are linked in a linear chain by α -(1, 4) glycosidic bonds, and the branching occurs by α -(1, 6) bonds. The different proportion of amylose and amylopectin affects the water absorption, viscosity and gelatinization degree of starch.

Amylopectin in the samples is removed by selective precipitant. Amylose and total starch are hydrolyzed into glucose. Glucose oxidase catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide. Peroxidase catalyzes the oxidation of 4-aminoantipyrine bisphenol by hydrogen peroxide to form colored compounds with characteristic absorption peaks at 510 nm.

Product composition:

Reagent	Volume	Storage
Extract solution I	Liquid 50mL \times 1	RT
Extract solution II	Self-supplied reagent	-
Reagent I	Liquid 15mL \times 1	2-8°C
Reagent II	Powder \times 1	20°C
Reagent III	Liquid 50mL \times 1	2-8°C
Reagent IVA	Liquid 0.1mL \times 1	2-8°C
Reagent IV B	Liquid 0.05mL \times 1	2-8°C
Reagent VA	Liquid 25mL \times 1	2-8°C
Reagent V B	Liquid 25mL \times 1	2-8°C
Standard	Powder \times 1	2-8°C

Preparation of solutions:

1. Extract solution I: The reagent is toxic. It is recommended to wear protective equipment during experimental in the sink cupboard. If the reagent solidifies, it can melt at 37 °C.
2. Extract solution II: Self-supplied 95% ethanol (about 50mL), store at room temperature. An empty brown 30mL bottle is provided in the kit. Please label the reagent name by yourself.
3. Reagent I working solution: Mix 3mL Reagent I and 7mL distilled water fully before use.
4. Reagent II: Dissolve with 6mL Reagent I working solution before use. Unused reagent can separate into small tubules and storage at -20°C for 2 weeks, avoid repeated freezing and thawing.
5. Reagent IV B: It is normal to appear turbid. Mix well before use.

6. Reagent IV: Reagent IV A and Reagent IV B need to be centrifuged and mixed fully before use. Mix Reagent III: Reagent IV A: Reagent IV B = 0.19mL: 0.02mL: 0.01 (0.22mL, 11T) according to sample number before use. It could be stored at -20°C for 2 weeks.
7. Reagent V: Mix Reagent VA: Reagent VB = 4mL: 4mL (8mL, 10T) according to sample number before use.
8. Standard: 10mg glucose. Add 1mL distilled water to fully dissolve and prepare 10mg/mL glucose standard solution before use. It could be stored at 2-8°C for 2 weeks.

Reagents and Equipment Required but Not Provided

Spectrophotometer/microplate reader, centrifuge, water bath, adjustable pipette, 1mL glass cuvette, mortar/grinding mill, 30-50 mesh sieve, 1.5mL tube, 2mL screw capped tube, ice, ethanol (>98%, AR) and distilled water.

Procedure:

I. Sample preparation:

1. Fresh samples are naturally air-dried or oven to dry at 37°C, then sieved by 30-50 mesh sieve.
2. Weigh 0.02g sample into a 2mL screw capped tube. Add 1mL Extract solution I and gently stir at low speed. Heat the tube for one minute in a boiling water bath and ensure that there is no gelatinous lumps of starch in the tube bottom (0.02g+2mL).
3. Mix well and heat it for 15minutes in a boiling water bath. Cool to room temperature after boiling.
4. Take 0.2mL solution of the previous step and 0.4mL Extract solution II into a new 2mL screw capped tube. Mix well and add 1mL Extract solution II. Stand at room temperature for 15minutes after mixing (containing 0.004g sample).
5. Centrifuge at 2000g for 5minutes at room temperature. Discard the supernatant and invert tube for 10minutes to drain Extract solution II. The leaving precipitation is used to determinate contents of amylose and total starch.
6. Add 0.4mL Extract solution I to the precipitation and mix gently. Heat the tube for 15 minutes in a boiling water bath. Cool to room temperature after boiling (0.004g+0.4mL).
7. Centrifuge at 2000g for 5minutes at room temperature. Take 0.1mL supernatant into a new 2mL screw capped tube. Add 1.15mL Reagent I working solution and mix well to prepare the Solution A (0.001g sample + (0.1mL+1.15mL), equal to 0.02g+25mL). It could be stored at 2-8°C for about one week and not be stored at -20°C.

Note: Using screw capped tube is to prevent the lid from bursting during boiling. It is better to make a hole in the lid and wrap the sealing film if using normal tube.

II. Determination procedure:

1. Preheat spectrophotometer for 30minutes, adjust wavelength to 510nm and set zero with distilled water.
2. Standard preparation: Dilute the standard to 0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125 mg/mL with Reagent III.
3. Pretreatment of amylose content detection: (add the following reagents successively into 1.5mL tube)

Reagent (mL)	Test Tube 1
Solution A	0.4
Reagent II	0.2
Reverse the tube to mix well (Do not vortex mixing). Stand at room temperature for one hour. Centrifuge at 14000g for 10minutes at room temperature.	
Supernatant	0.2
Reagent III	0.6
Boiling for 5minutes, incubate at 40°C for 5minutes.	
Reagent IV	0.02
Incubate at 40°C for 30minutes and centrifuge at 2000g for 5minutes at room temperature. Take the supernatant as Solution B for amylose content detection	

4. Pretreatment of total starch content detection: (add the following reagents successively into 1.5mL tube).

Reagent (mL)	Test Tube2
Solution A	0.1
Reagent III	0.8
Incubate at 40°C for 5minutes.	
Reagent IV	0.02
Incubate at 40°C for 10 minutes and centrifuge at 2000g for 5minutes at room temperature. Take the supernatant as Solution C for total starch content detection.	

5. Content detection: (add the following reagents successively into 1.5mL tube)

Reagent (μL)	Test Tube 1	Test Tube 2	Blank Tube	Standard Tube
Solution B	0.2	-	-	-
Solution C	-	0.2	-	-
Reagent III	-	-	0.2	-
Standard	-	-	-	0.2
Reagent V	0.8	0.8	0.8	0.8
Incubate at 40°C for 20 minutes. Detect the absorbance value of each tube at 510nm and record as A_{T1} , A_{T2} , A_B and A_S . Calculate $\Delta A_{T1} = A_{T1} - A_B$, $\Delta A_{T2} = A_{T2} - A_B$, $\Delta A_S = A_S - A_B$. The blank and standard curve only need to be measured 1-2 times.				

III. Calculations:

1. Standard curve

The concentration of standard solution as x-axis, ΔA_S as y-axis, obtain the equation $y = kx + b$.

Take ΔA_{T1} and ΔA_{T2} to the equation to acquire $(x_1, \text{mg/mL})$ and $(x_2, \text{mg/mL})$.

2. Content calculations:

$$\text{Amylose content (mg/g weight)} = (x_1 \times V_1 \div V_2 \times V_3 \div V_4) \times V_E \div 1.11 \div W \times F = 138.51 \times x_1 \div W \times F$$

$$\text{Total starch content (mg/g weight)} = (x_2 \times V_1 \div V_2 \times V_E) \div 1.11 \div W \times F = 207.21 \times x_2 \div W \times F$$

$$\text{Amylopectin content (mg/g weight)} = \text{Total starch content} - \text{Amylose content}$$

V_1 : Volume of Solution B, 0.41mL; V_2 : Volume of supernatant in the pretreatment of amylase content detection, 0.1mL; V_3 : Volume of reaction mixture at room temperature in the pretreatment of amylose content detection, 0.3mL; V_4 : Volume of added Solution A in the pretreatment of amylose content detection, 0.2mL; V_5 : Volume of reaction mixture in the pretreatment of total starch content detection, 0.46mL; V_6 : Volume of added Solution A in the pretreatment of total starch content detection, 0.05mL; V_E : Volume of produced Solution A after sample preparation, 25mL; 1.11: It is the constant of converting glucose content into starch content; W: sample weight, g; F: Dilution factor.

3. Rate calculation of amylose/amylopectin in the sample

$$\text{Amylose content (\%)} = \text{Amylose content} \div \text{Total starch content} \times 100\%$$

$$\text{Amylopectin content (\%)} = (\text{Total starch content} - \text{Amylose content}) \div \text{Total starch content} \times 100\%$$

Note:

1. It is normal to produce starch precipitation in the sample preparation after adding Extract solution II.
2. It is normal to produce flocculent precipitate after boiling in the pretreatment of amylase content detection.

3. If ΔA_T is more than 0.8, it is recommended to dilute Solution A with distilled water before detection.
4. If ΔA_T is less than 0.01, it is recommended to reduce volume of added Reagent I working solution in the sample preparation.
5. This kit could be used to detect amylose content and total starch content of 48 samples (100T/48S).