Amylose/Amylopectin/Total Starch Content Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC6105

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent	Size	Preservation Condition
Extract solution I	Liquid 85mL×1	RT
Extract solution II	Self-supplied reagent	-
Reagent I	Liquid 25mL×1	2-8℃
Reagent II	Powder ×1	-20℃
Reagent III	Liquid 50mL×1	2-8℃
Reagent IVA	Liquid 0.1mL×1	2-8℃
Reagent IVB	Liquid 0.05mL×1	2-8℃
Reagent VA	Liquid 25mL×1	2-8℃
Reagent VB	Liquid 25mL×1	2-8℃
Standard	Powder ×1	2-8℃

Solution Preparation:

1. Extract solution I: The reagent is toxic. It is recommended to wear protective equipment during experimental in the stink cupboard. If the reagent solidifies, it can melt at 37 °C.
2. Extract solution II: Self-supplied 95% ethanol (about 85mL), 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 6 mL Reagent I working solution before use. Unused reagent can separate into small tubules and storage at -20℃ for 2 weeks, avoid repeated freezing and thawing.
5. Reagent IVB: It is normal to appear turbid. Mix well before use.
6. Reagent IV: Reagent IVA and Reagent IVB need to be centrifuged and mixed fully before use. Mix Reagent III: Reagent IVA: Reagent IVB =0.19mL: 0.02mL: 0.01 (0.22mL, 22T) according to sample number before use. It could be stored at -20℃ for 2 weeks.
7. Reagent V: Mix Reagent VA: Reagent VB =4mL: 4mL (8mL, 20T) according to sample number before use.
8. Standard: 10mg glucose. Add 1 mL distilled water to fully dissolve and prepare 10 mg/mL glucose standard solution before use. It could be stored at 2-8℃ for 2 weeks.

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.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, centrifuge, water bath, adjustable pipette, micro glass cuvette/96 well plate, 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℃, 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 15 minutes 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 15min after mixing (containing 0.004g sample).
5. Centrifuge at 2000g for 5 minutes at room temperature. Discard the supernatant and invert tube for 10 minutes 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 5 minutes 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 (0.001gsample+(0.1mL+1.15mL), equal to 0.02g+25mL). It could be stored at 2-8℃ for about one weak and not be stored at -20℃.

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/microplate reader for 30 minutes, adjust wavelength to 510 nm 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 Tube1
Solution A	0.2
Reagent II	0.1
Reverse the tube to mix well (**Do not vortex mixing**). Stand at room temperature for one hour. Centrifuge at 14000g for 10 minutes at room temperature.	

Supernatant	0.1

Reagent III	0.3
Boiling for 5min, incubate at 40℃ for 5 minutes.	
Reagent IV	0.01
Incubate at 40℃ for 30 minutes and centrifuge at 2000g for 5 minutes 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	0.05
Reagent III	0.4
Incubate at 40℃ for 5 minutes.	
Reagent IV	0.01
Incubate at 40℃ for 10 minutes and centrifuge at 2000g for 5 minutes 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 (mL)	Test Tube1	Test Tube2	Blank tube	Standard tube
Solution B	0.1	-	-	-
Solution C	-	0.1	-	-
Reagent III	-	-	0.1	-
Standard	-	-	-	0.1
Reagent V	0.4	0.4	0.4	0.4
Incubate at 40℃ for 20 minutes. Detect the absorbance value of each tube at 510 nm 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, mg/mL) and (x_2, mg/mL).

2. Content calculations:

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

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

Amylopectin content (mg/g weight) = Total starch content - Amylose content

V_1: Volume of Solution B, 0.41mL; V_2: Volume of supernatant in the pretreatment of amylose 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

Amylose content (%) = Amylose content ÷Total starch content×100%

Amylopectin content (%) = (Total starch content - Amylose content) ÷Total starch content×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 amylose 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).

Experimental example:

1. Take 0.0202g of wheat flour sample for sample processing and follow the determination steps to measure and calculate $\Delta A_{T1}=A_{T1}-A_B=0.118-0.049=0.069$, $\Delta A_{T2}=A_{T2}-A_B=0.266-0.049=0.217$. Bring the result into the standard curve y=4.284x+0.001 and calculate $x_1=0.016$ and $x_2=0.050$. The result is calculated according to the sample weight:

Amylose content (mg/g weight) =$138.51\times x_1 \div W\times F$ =109.71 mg/g weight.

Total starch content (mg/g weight) =$207.21\times x_2 \div W\times F$ =512.90 mg/g weight.

Amylopectin content (mg/g weight) = Total starch content - Amylose content=403.19 mg/g weight.

Amylose content (%) = Amylose content ÷Total starch content×100%=21.4%.

Amylopectin content (%) = (Total starch content - Amylose content) ÷Total starch content ×100%= 78.6%.

2. Take 0.0199g of green gram starch sample for sample processing and follow the determination steps to measure and calculate $\Delta A_{T1}=A_{T1}-A_B=0.068-0.049=0.019$, $\Delta A_{T2}=A_{T2}-A_B=0.140-0.049=0.091$. Bring the result into the standard curve y=4.284x+0.001 and calculate $x_1=0.004$ and $x_2=0.021$. The result is calculated according to the sample weight:

Amylose content (mg/g weight) =$138.51\times x_1 \div W\times F$ =27.84 mg/g weight.

Total starch content (mg/g weight) =$207.21\times x_2 \div W\times F$ =218.66 mg/g weight.

Amylopectin content (mg/g weight) = Total starch content - Amylose content=190.82 mg/g weight.

Amylose content (%) = Amylose content ÷Total starch content×100%=12.7%.

Amylopectin content (%) = (Total starch content - Amylose content) ÷Total starch content ×100%= 87.3%.

References：

[1] Norman K Matheson, Lynsey A Welsh. Estimation and fractionation of the essentially unbranched (amylose) and branched (amylopectin) components of starches with concanavalin A. Carbohydrate Research, 1988, 180(2): 301-313.

[2] Yun S H, Norman K Matheson. Estimation of Amylose Content of Starches after Precipitation of Amylopectin by Concanavalin-A. Starch-starke, 1990, 42: 302-305.

Related Products：

BC2040/BC2045	β-amylase(β-AL) Activity Assay Kit
BC0430/BC0435	ADPG Pyrophosphorylase (AGP) Activity Assay Kit

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