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Resistant/Non-Resistant 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: AK0951-100T-48S
Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle.

Reagent Name	Size	Preservation Condition
Reagent I	Liquid 50mL×1	2-8℃
Reagent II	Powder ×1	-20℃
Reagent III	Liquid 50mL×1	2-8℃
Reagent IV	Liquid 50mL×1	2-8℃
Reagent V	Liquid 120mL×1	2-8℃
Reagent VI	Liquid 60mL×1	2-8℃
Reagent VII	Liquid 0.84mL×1	2-8℃
Reagent VIIIA	Liquid 20mL×1	2-8℃
Reagent VIIIB	Liquid 20mL×1	2-8℃
Standard	Powder ×1	2-8℃

Solution Preparation:

1. Reagent II: Dissolve with 6 mL of Reagent I before use. Unused reagent can separate into small tubules and storage at -20℃ for 4 weeks, avoid repeated freezing and thawing.
2. Reagent VIII: Mix Reagent VIIIA: Reagent VIIIB =3mL: 3mL (6mL, 20T) according to sample number before use.
3. 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 4 weeks.
4. Preparation of 1mg/mL standard solution: Mix 0.1mL 10mg/mL glucose standard solution and 0.9mL distilled water to prepare 1mg/mL standard solution.

Product Description:

Resistant starch (RS) is that portion of the starch that cannot be digested and absorbed in the small intestine of healthy human. RS is partially or completely fermented in the colon, producing many short-chain fatty acids, which can inhibit the hyperproliferation of intestinal epithelial cells, reduce intestinal inflammation, and reduce the risk of colon cancer. RS has good physicochemical and functional properties and is widely used in the production and processing of various foods to improve product quality and nutritional structure.

RS in the sample is prepared after α-amylase and amyloglucosidase hydrolyze non-resistant starch into glucose. RS is also 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, mortar/grinding mill, 30-50 mesh sieve, balance, centrifuge, shaking water bath, water bath/metal bath, magnetic stirrer, magnetic stirrer bars, adjustable pipette, micro glass cuvette/96 well plate, 2mL tube, 5mL tube, 50mL tube, ice 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.

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 510 nm and set zero with distilled water.
2. Precool Reagent V at 2-8℃ for 30 minutes.
3. Hydrolysis of non-resistant starch: (add the following reagents successively into 2ml tube)

Reagent（mL）	Test Tube1
Sample	0.02g
Reagent I	0.7
Mix well and preheat at 37℃ for 5 minutes	
Reagent II	0.1
Tightly cap the tube, incubate at 37℃ with continuous shaking (200 strokes/min) for 4 hours. Note: Do not mix on a vortex mixer as this may affect starch hydrolysis. The reaction needs shaking water bath or other instrument that can shake in liner motion.	
Reagent III	0.8
Mix well and Centrifuge at 4000rpm for 10 minutes at room temperature. Take the supernatant in a 5mL tube, add Reagent IV to the precipitate.	
Reagent IV	0.8
Mix well and Centrifuge at 4000rpm for 10 minutes at room temperature. Take the supernatant in the 5mL tube and mix all the supernatant (about 2.4mL) for the determination of non-resistant starch content. The precipitate is used for the determination of RS content.	

4. Hydrolysis of RS:

Reagent（mL）	Test Tube2
Sample	All precipitate
Precooled Reagent V	2
Dissolve the precipitate and transfer into a 50mL tube. stir at 200rpm for 20 min in an ice bath over a magnetic stirrer. Take 250 μL the RS solution for the next reaction. Note: A. There may be transparent gel insoluble in the RS solution before stirring. It is clear and no insoluble in the RS solution after stirring. B. It can improve efficiency if put multiple 50mL tube in a large container to stir.	
The RS solution	0.25
Reagent VI	1

Mix well.	
Reagent VII	0.014
Mix well and incubate at 50℃ for 30 minutes. Centrifuge at 13000rpm for 5 minutes at room temperature. Take the supernatant for the determination of RS content.	

5. Content detection:

Reagent (mL)	Test Tube1	Test Tube2	Blank tube	Standard tube
Hydrolysis supernatant of non-resistant starch	0.01	-	-	-
Hydrolysis supernatant of RS	-	0.01	-	-
Distilled water	-	-	0.01	-
Standard	-	-	-	0.01
Reagent VIII	0.3	0.3	0.3	0.3
Mix well and incubate at 50℃ for 30 minutes. Take 200μL mixture into micro glass cuvette/ 96 well plate and 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 tubes only need to be measured 1-2 times.				

III. Calculations:

1. Content calculations:

Non-resistant starch content (mg/g weight) = $(\Delta A_{T1} \div \Delta A_S \times C_S) \times V4 \div 1.11 \div W \times F$

$= 2.162 \times \Delta A_{T1} \div \Delta A_S \div W \times F$

RS content (mg/g weight) = $(\Delta A_{T2} \div \Delta A_S \times C_S) \times V1 \div (W \div V3 \times V2) \div 1.11 \times F$

$= 9.110 \times \Delta A_{T2} \div \Delta A_S \div W \times F$

Starch content (mg/g weight) = RS content +Non-resistant starch content

C_S: Concentration of standard, 1mg/mL; V1: reaction volume of RS hydrolysis, 1.264mL; V2: Added volume of the RS solution in the RS hydrolysis, 0.25mL; V3: Total volume of the RS solution, 2mL; V4: Total volume of non-resistant starch hydrolysis supernatant, 2.4mL; 1.11: It is the constant of converting glucose content into starch content; W: sample weight, g; F: Dilution factor of the RS solution/ non-resistant starch hydrolysis supernatant.

2. Rate calculation of RS/ non-resistant starch in the sample

Non-resistant starch percentage content (%) = Non-resistant starch content÷1000×100%

RS percentage content (%) = RS content÷1000×100%

1000: Unit conversion factor, 1000mg=1g.

Note:

1. If ΔA_{T1} is more than ΔA_S, it is recommended to dilute non-resistant starch hydrolysis supernatant with distilled water before determination. And modify the calculation formula.
2. If ΔA_{T2} is more than ΔA_S, it is recommended to dilute the RS solution with Reagent V before determination. And modify the calculation formula.

3. This kit could be used to detect RS content and non-resistant starch content of 48 samples (100T/48S).

Experimental example:

1. Take 0.02g of potato starch sample for sample processing, dilute non-resistant starch hydrolysis supernatant 4 times with distilled water and follow the determination steps to measure and calculate $\Delta A_{T1}=A_{T1}-A_B=0.572-0.053=0.519$, $\Delta A_{T2}=A_{T2}-A_B=0.749-0.053=0.696$, $\Delta A_S=A_S-A_B=0.764-0.053=0.711$. Calculate the content according to the sample weight:

   Non-resistant starch content (mg/g weight) $=2.162\times\Delta A_{T1}\div\Delta A_S\div W\times F=315.634$ mg/g weight.

   RS content (mg/g weight) $=9.110\times\Delta A_{T2}\div\Delta A_S\div W\times F=445.89$ mg/g weight.

   Starch content (mg/g weight) = RS content +Non-resistant starch content=761.524 mg/g weight.

   Non-resistant starch percentage content (%) = Non-resistant starch content÷1000×100%=32%.

   RS percentage content (%) = RS content÷1000×100%=45%.

2. Take 0.02g of wheat starch sample for sample processing, dilute non-resistant starch hydrolysis supernatant 4 times with distilled water, then follow the determination steps to measure and calculate $\Delta A_{T1}=A_{T1}-A_B=0.420-0.053=0.367$, $\Delta A_{T2}=A_{T2}-A_B=0.406-0.053=0.353$, $\Delta A_S=A_S-A_B=0.764-0.053=0.711$. Calculate the content according to the sample weight:

   Non-resistant starch content (mg/g weight) $=2.162\times\Delta A_{T1}\div\Delta A_S\div W\times F=223.194$ mg/g weight.

   RS content (mg/g weight) $=9.110\times\Delta A_{T2}\div\Delta A_S\div W\times F=226.148$ mg/g weight.

   Starch content (mg/g weight) = RS content +Non-resistant starch content=449.342 mg/g weight.

   Non-resistant starch percentage content (%) = Non-resistant starch content÷1000×100%=22%.

   RS percentage content (%) = RS content÷1000×100%=23%.

References:

[1] Radhiah Shukri, Lijia Zhu, Paul A, et al. Seib. Direct in-vitro assay of resistant starch in phosphorylated cross-linked starch[J]. Bioactive Carbohydrates and Dietary Fibre, 2015, 5(1): 1-9.

[2] Barry V. McCleary, Naomi Sloane, Anna Draga. Determination of total dietary fibre and available carbohydrates: A rapid integrated procedure that simulates in vivo digestion[J]. Starch-starke, 2015, 67: 860-883.

Related Products:

AK0243/AK0244	β-amylase(β-AL) Activity Assay Kit
AK0320/AK0321	ADPG Pyrophosphorylase (AGP) Activity Assay Kit
AK0095/AK0096	Starch Debranching Enzyme (DBE) Activity Assay Kit