Phytic Acid Content Assay Kit

Detection Equipment: Spectrophotometer

Catalog Number: BC5840

Size: 50T/24S

Components:Please carefully check the volume of the reagent and the volume in the bottle before use. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation condition
Extract I	Liquid 60 mL×1	2-8℃ storage
Extract II	Liquid 10 mL×1	2-8℃ storage
Reagent I	Liquid 20 mL×1	2-8℃ storage
Reagent II	Powder ×1	-20℃ storage
Reagent III	Powder ×1	2-8℃ storage
Reagent IV	Powder ×1	2-8℃ storage
Standard	Powder ×1	2-8℃ storage

Solution preparation :

1. Reagent II: add 8mL of Reagent I before use, fully dissolve; The reagents that could not be used up were storage at -20℃ for 4 weeks to avoid repeated freezing and thawing.
2. Reagent III: add 5.9mL distilled water to fully dissolve before use, and then slowly add 1.6mL concentrated sulfuric acid under the liquid level to fully mix; It can be storage for 4 weeks at 2-8℃.
3. Reagent IV: before use, add 37.5mL distilled water to fully dissolve, and then slowly add 37.5μL concentrated sulfuric acid under the liquid level to fully mix; It can be storage for 4 weeks at 2-8℃.
4. Working solution: according to the sample volume before use, according to the ratio of Reagent III: Reagent IV =1mL: 5mL (a total of 6mL, 8T), mix, use up on the day after configuration;
5. Standard: Add 1.08mL of Reagent I to fully dissolve, prepare 10μmol/mL phytic acid Standard , and storage at 2-8℃ for 4 weeks;
6. Preparation of 125nmol/mL Standard: before clinical use, 50μL 10μmol/mL phytic acid standard and 450μL distilled water were mixed into 1μmol/mL Standard (1000nmol/mL); Another 125μL of 1000nmol/mL Standard and 875μL of distilled water were mixed to make 125nmol/mL phytic acid standard, which was used for the following Standard tube determination in the operating table and is now prepared for use.

Description:

Phytic acid, also known as inositol hexadecanol hexophosphorate, is ubiquitous in eukaryotic cells and plays a key role in many cellular activities, such as maintaining inorganic phosphorus homeostasis, participating in plant hormone signaling, and acting as a cofactor for enzymes involved in DNA repair, RNA editing, and mRNA export. Phytic acid (PA) is widely present in plant foods such as cereals, legumes, fruits, vegetables and dried fruits and provides the main phosphorus source during plant germination and growth.

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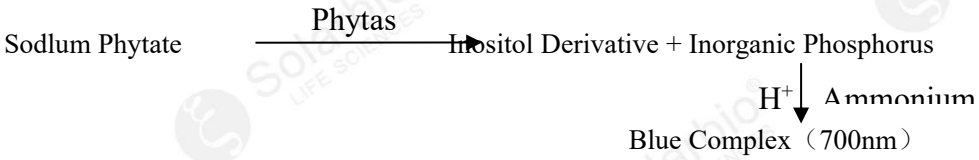
Under certain environmental conditions, phytase can decomcause sodium phytate (sodium dodecyl inositol hexaphosphosponate) to produce inorganic phosphorus and inositol derivatives. Under acidic conditions, inorganic phosphorus reacts with ammonium molybdate chromogenic agent to produce blue molybdenum blue substance, which has a characteristic absorption peak at 700nm. By measuring the content of inorganic phosphorus, phytic acid content can be calculated.



Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, cryogenic centrifuge, water bath/thermostatic incubator, 1mL glass cuvette, analytical balance, adjustable pipet gun, mortar/homogenizer, concentrated sulfuric acid (>98%, AR), ice and distilled water.

Protocol:

I. Sample processing (The sample size to be tested can be appropriately adjusted, and the specific proportion can be referred to the literature)

1. **Fresh plant sample:** According to the mass (g) : Extract I volume (mL) is 1:5~10 (it is recommended to weigh about 0.2g and add 2mL Extract I). Add Extract I, fully homogenize, shake at room temperature for 2h, then centrifuge at 4℃, 10000g for 10min, take 1.6mL supernatant, slowly add 0.3mL Extract II, slowly blow and mix until no bubbles occur. After centrifugation at 4℃ 10000g for 10min, the supernatant was taken to be measured.
2. **Dry powder plant sample:** According to the mass (g) : Extract I volume (mL) is 1:5-20 (it is recommended to weigh about 0.1g and add 2mL Extract I). Add Extract I, fully homogenize, shake at room temperature for 2h, then centrifuge at 4℃, 10000g for 10min, take 1.6mL supernatant, slowly add 0.3mL Extract II, slowly blow and mix until no bubbles occur. After centrifugation at 4℃ 10000g for 10min, the supernatant was taken to be measured.
3. **Liquid sample:** Take 200μL liquid and add 2mL Extract I, shake at room temperature for 2h, then centrifuge at 10000g for 10min at 4℃, take 1.6mL supernatant, then slowly add 0.3mL Extract II, slowly blow and mix until no bubbles are generated, centrifuged at 10000g for 10min at 4℃, take the supernatant to be tested.

Note: Extract II needs to be added slowly, and a large number of bubbles will be produced after addition, so it is recommended to use 5 mL EP tubes for operation.

II. Measurement Steps

1. It can be seen that the spectrophotometer is preheated for more than 30min, the wavelength is adjusted to 700nm, and zero it with distilled water.

2. Operation table (Add the following reagents to the 2mL EP tube) :

Reagent name（μL）	Test tube	Control tube	Standard tube	Blank tube
Sample	600	600	-	-
Standard	-	-	600	-
Reagent I	-	250	-	600
Reagent II	250	-	250	250
Water bath at 37℃ for 30min				
Working solution	750	750	750	750
After standing at room temperature for 10min, the absorbance value was measured at 700nm, respectively recorded as A_{text}, $A_{control}$, A_{blank} and $A_{standard}$, calculate $\Delta A_{text}=A_{text}-A_{control}$, $\Delta A_{standard}=A_{standard}-A_{blank}$. (Blank tube and standard tube only need to be tested 1-2 times.)				

III. Calculations

1. Calculated according to the protein concentration of the sample

Phytate acid content (nmol/mg prot) $=\Delta A_{text} \times C_{standard} \div \Delta A_{standard} \times V_{sample} \div (V_{sample} \times Cpr) = 125 \times \Delta A_{text} \div \Delta A_{standard} \div Cpr$

2. Calculated according to sample quality

Phytic acid content (nmol/g mass) $=\Delta A_{text} \times C_{standard} \div \Delta A_{standard} \times (V_{supernatant} + V_{Extract\ II}) \div (W \times V_{supernatant} \div V_{Extract\ I}) = 296.875 \times \Delta A_{text} \div \Delta A_{standard} \div W$

3. Calculate by liquid volume

Phytic acid content (nmol/mL) $= \Delta A_{text} \times C_{standard} \div \Delta A_{standard} \times (V_{supernatant} + V_{Extract\ II}) \div [V_{liquid} \times V_{supernatant} \div (V_{Extract\ I} + V_{liquid})] = 1632.81 \times \Delta A_{text} \div \Delta A_{standard}$

$C_{standard}$: Standard tube concentration, 125nmol/mL;

V_{sample}: Added sample volume, 0.6mL;

$V_{supernatant}$: Volume of supernatant during extraction, 1.6mL;

$V_{Extract\ II}$: Add the volume of extract II, 0.3mL;

$V_{Extract\ I}$: Volume of added extract one, 2mL;

Cpr: Sample protein concentration, mg/mL;

V_{liquid}: Liquid sample volume, 0.2mL;

W: Sample quality, g.

Note:

1. If ΔA is less than 0.010 or the absorbance value of the measuring tube is close to the blank tube, the sample size can be increased before the measurement; If ΔA is determined to be greater than 1, it is recommended that the sample supernatant be appropriately diluted with distilled water before the assay is performed. Note that the calculation formula is modified synchronously.

2. If the sample is turbid after adding the working solution, it is recommended to dilute the sample supernatant with distilled water before the determination. Note that the calculation formula is modified

synchronously.

3, Extract I contained a protein precipitant, so the supernatant could not be used for protein concentration determination. Additional sample were taken if protein content was to be determined.

Experimental example:

1. Weigh 0.2089g onion sample, follow the extraction steps and determination steps, and calculate with 1mL glass cuvette: $\Delta A_{text} = A_{text} - A_{control} = 0.595-0.530=0.065$, $\Delta A_{standard} = A_{standard} - A_{blank} = 0.741-0.213=0.528$, calculated according to the sample quality:

Phytic acid content (nmol/g mass) $= 296.875 \times \Delta A_{text} \div \Delta A_{standard} \div W = 174.950$ nmol/g mass.

2. Weigh 0.1001g of wheat flour, follow the extraction steps and determination steps, and calculate with A 1mL glass cuvette: $\Delta A_{text} = A_{text} - A_{control} = 0.521-0.238=0.283$, $\Delta A_{standard} = A_{standard} - A_{blank} = 0.741-0.213=0.528$, and calculate according to the sample quality:

Phytic acid content (nmol/g mass) $= 296.875 \times \Delta A_{text} \div \Delta A_{standard} \div W = 1589.62$ nmol/g mass.

References:

[1] Senna R, Simonin V, Silva-Neto M A C, et al. Induction of acid phosphatase activity during germination of maize (Zea mays) seeds.[J]. Plant Physiology & Biochemistry, 2006, 44(7-9):467-473.

[2] Iqbal T H, Lewis K O, Cooper BT . Phytase activity in the human and rat small intestine[J]. Gut, 1994, 35(9):1233-1236.

[3] Azeke M A, Egielewa S J, Ihimire E. Effect of germination on the phytase activity, phytate and total phosphorus contents of rice (Oryza sativa), maize (Zea mays), millet (Panicum miliaceum), sorghum (Sorghum bicolor) and wheat (Triticum aestivum)[J]. Journal of Food Science&Technology, 2011.

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