Phytic Acid Content Assay Kit

Detection Equipment: Spectrophotometer/Microplate Reader

Catalog Number: BC5845

Size: 100T/48S

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 10 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

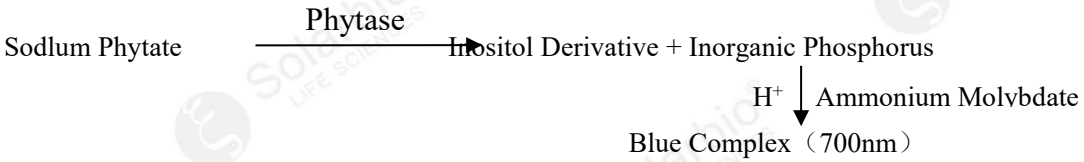
Solution reparation :

1. Reagent II: add 4mL Reagent I before clinical 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: before use, add 2.36mL distilled water to fully dissolve, and then extend the tip of the gun under the liquid level and slowly add 0.64mL concentrated sulfuric acid to fully mix; It can be storage for 4 weeks at 2-8℃.
3. Reagent IV: before use, add 15mL distilled water to fully dissolve, and then slowly add 15μL 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, 40T), mix, use up the same 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 250nmol/mL Standard: before clinical use, 50μL 10μmol/mL phytic acid standard and 450μL distilled water were mixed into 1μmol/mL Standard (1000nmol/mL); Another 250μL of 1000nmol/mL Standard and 750μL of distilled water were mixed to prepare 250nmol/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.

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/microplate reader, cryogenic centrifuge, water bath/constant temperature incubator, analytical balance, micro glass cuvette/96 well flat-bottom plate, 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 0.1g and add 1mL Extract I). Add Extract I, fully homogenize, shake at room temperature for 2h, then centrifuge at 4℃, 10000g for 10min, take 0.8mL supernatant, slowly add 0.15mL 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:** aAccording to the mass (g) : Extract I volume (mL) is 1:5-20 (it is recommended to weigh about 0.05g and add 1mL Extract I). Add Extract I, fully homogenize, shake at room temperature for 2h, then centrifuge at 4℃, 10000g for 10min, take 0.8mL supernatant, slowly add 0.15mL 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 100μL liquid and add 1mL Extract I, shake at room temperature for 2h, then centrifuge at 10000g for 10min at 4℃, take 0.8mL supernatant, then slowly add 0.15mL 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 1.5mL 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 1.5mL EP tube) :

Reagent name（μL）	Test tube	Control tube	Standard tube	Blank tube
Sample	120	120	-	-

Standard	-	-	120	-
Reagent I	-	50	-	120
Reagent II	50	-	50	50
Water bath at 37℃ for 30min				
Working solution	150	150	150	150
After standing at room temperature for 10min, absorb 0.2mL reaction solution, the absorbance value was measured at 700nm, respectively recorded as A_{text}, $A_{control}$, A_{blank} and $A_{standard}$, calculate $\Delta A_{text}=A_{control}-A_{text}$, $\Delta A_{standard}=A_{standard}-A_{blank}$. (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) $=\Delta A_{text} \times C_{standard} \div \Delta A_{standard} \times V_{sample} \div (V_{sample} \times Cpr) = 250 \times \Delta A_{text} \div \Delta A_{standard} \div Cpr$

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) $= \Delta A_{text} \times C_{standard} \div \Delta A_{standard} \times (V_{supernatant} + V_{Extract\ II}) \div [V_{liquid} \times V_{supernatant} \div (V_{Extract\ I} + V_{liquid})] = 3265.625 \times \Delta A_{text} \div \Delta A_{standard}$

$C_{standard}$: Standard tube concentration, 250nmol/mL;

V_{sample}: Added sample volume, 0.12mL;

$V_{supernatant}$: Volume of supernatant during extraction, 0.8mL;

$V_{Extract\ II}$: Add the volume of extract II, 0.15mL;

$V_{Extract\ I}$: Volume of added extract one, 1mL;

Cpr: Sample protein concentration, mg/mL;

V_{liquid}: Liquid sample volume, 0.1mL;

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 0.1044g onion sample, follow the extraction steps and determination steps, and calculate with 96 well flat-bottom plate: $\Delta A_{text} = A_{text} - A_{control} = 0.411-0.375=0.036$, $\Delta A_{standard} = A_{standard} - A_{blank} = 0.811-0.096=0.715$, calculated according to the sample quality:
   Phytic acid content (nmol/g mass) $= 296.875 \times \Delta A_{text} \div \Delta A_{standard} \div W = 143.18$ nmol/g mass.
2. Weigh 0.05g of wheat flour, follow the extraction steps and determination steps, and calculate with 96 well flat-bottom plate: $\Delta A_{text} = A_{text} - A_{control} = 0.331-0.188=0.143$, $\Delta A_{standard} = A_{standard} - A_{blank} = 0.811-0.096=0.715$, and calculate according to the sample quality:
   Phytic acid content (nmol/g mass) $= 296.875 \times \Delta A_{text} \div \Delta A_{standard} \div W = 1187.5$ nmol/g mass.

References：

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[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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