EZMeta™ Tissue Inorganic Phosphorus Colorimetric Assay Kit

Cat #: D-AKC2170

Size: 96T

Storage: Stored at 4°C for 12 months, protected from light

Product Information

Applicable samples: Animal and Plant Tissue

Assay Principle

Inorganic phosphorus mainly refers to inorganic phosphate radicals, which participate in various metabolic processes in organisms, including energy metabolism, nucleic acid metabolism, protein phosphorylation and dephosphorylation, and can also promote the synthesis, transformation, and transportation of carbohydrates. Ammonium molybdate reacts with inorganic phosphate to form a substance with a characteristic absorption peak at 660 nm. By measuring the absorbance at 660 nm, the inorganic phosphorus content can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
Reagent Ⅰ	100 mL	4°C
Reagent Ⅱ	5 mL	4°C
Reagent Ⅲ	1	4°C, protected from light
Reagent Ⅳ	1	4°C, protected from light
Standard	1 mL	4°C

Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at 660 nm

·96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

·adjustable water bath, centrifuge

·Deionized water, homogenizer

Reagent Preparation

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent III: Prepare before use, add 2 mL of deionized water and fully dissolve before use. Store at 4°C, protected from light.

Reagent IV: Prepare before use, add 8 mL of deionized water and fully dissolve before use. Store at 4°C, protected from light.

Phosphorus Fixative: Prepare according to the ratio of deionized water: Reagent II: Reagent III: Reagent IV=10:5:2:8 (Please prepare according to actual needs), and use on the same day.

Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Sample Preparation

1.Tissues samples: Weigh 0.1 g tissue, add 1 mL Reagent Ⅰ and homogenize on ice (Tissue mass (g): Reagent I volume (mL) is 1:10). Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 660 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (the following operations are performed in a 1.5 mL centrifuge tube):

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Supernatant	0	0	10
Deionized Water	100	90	90
Standard	0	10	0
Phosphorus Fixative	100	100	100

3. After mixing, place it in a 40°C water bath for 10 min, cool it at room temperature for 10 min,and take 200 μL supernatant add into visible spectrophotometer or 96-well plate, and then measure the absorbance at 660 nm. The absorbance of blank well, standard well, test well recorded as A_{Blank}, $A_{Standard}$ and A_{Test}. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Blank well and standard well only need to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. Increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with Reagent Ⅰ, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

(1) Calculated by protein concentration

Inorganic phosphorus content (μmol/mg prot)=$(C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard})\times V_{Total}\div$ Cpr=$\mathbf{1\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr}$

(2) Calculated by sample fresh weight

Inorganic phosphorus content (μmol/g fresh weight)=$(C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard})\times V_{Total}\div W=\mathbf{1\times\Delta A_{Test}\div\Delta A_{Standard}\div W}$

$C_{Standard}$: 1 mmol/L; V_{Total}: total volume of supernatant, 1 mL=0.001 L; W: sample weight, g.

Typical Data

Experimental examples:

1.Take 0.1g of green pineapple and add 1 mL of Reagent Ⅰ. Centrifuge the supernatant and follow the measurement steps. Use a 96 well plate to measure A_{Test}=0.334, A_{Blank}=0.043, and $A_{Standard}$=0.311. Calculate the inorganic phosphorus content based on the sample mass to obtain:

inorganic phosphorus content（μmol/g sample)=1×ΔA_{Test}÷$\Delta A_{Standard}$÷0.1=10.86 μmol/g sample.

2.Take 0.04 g of mouse muscle and add 0.4 mL of Reagent Ⅰ. Centrifuge the supernatant and follow the measurement steps. Use a 96 well plate to measure A_{Test}=1.201 , A_{Blank}=0.042, and $A_{Standard}$=0.29 . Calculate the inorganic phosphorus content based on the sample mass to obtain:

inorganic phosphorus content（μmol/g sample)=0.4×ΔA_{Test}÷$\Delta A_{Standard}$÷0.04=46.73 μmol/g sample.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.