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Vitamin B1 (VB1) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Detection equipment: Spectrophotometer/ Microplate reader

Cat No: AK0343

Size: 100T/96S

Components:

Extract solution: Liquid 70 mL×1. Store at 4℃ . Contains insoluble matter, shake well before use.

Diluent solution: Liquid 100 mL×1. Store at 4℃ .

Reagent I: Liquid 2.5 mL×1. Store at 4℃ .

Reagent II: Liquid 3 mL×1. Store at 4℃ and protect from light.

Reagent III: Liquid 3 mL×1. Store at 4℃ .

Reagent IV: Liquid 7 mL×1. Store at 4℃ .

Reagent V: Liquid 4 mL×1. Store at 4℃ and protect from light.

Standard: Powder×1, 10 mg Vitamin B1. Store at 4℃ and protect from light. Dissolve it with 1 mL of Diluent solution to prepare as 10 mg/mL (10000 μg/mL) standard solution before use.

Description:

Vitamin B1 (VB1), also known as thiamin, is involved in the catabolism of sugars in the form of coenzyme and plays an important role in energy metabolism.

Under alkaline conditions, VB1 reduces potassium ferricyanide to form potassium ferrocyanide. Potassium ferrocyanide and Fe^{3+} form Prussian blue under weak acid conditions. The absorbance ratio of Bruce blue is in direct proportion to the contents of VB1.

Required but not provided:

Spectrophotometer/microplate reader, centrifuge, transferpettor, water bath, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, EP tubes, ice and distilled water.

Protocol:

I. Sample preparation

1. Tissue

Homogenate the tissue samples and according to the ratio of tissue sample weight (g) and extract solution volume (mL) is 1:5~10 to add the extract solution (It is recommended to add 0.6 mL of extraction solution to 0.1 g of crushed tissue sample), then extract for 30 minutes at 60℃. After extraction, add 0.4 mL of distilled water and centrifuge at 13000×g for 10 minutes at 25℃ to remove insoluble materials and take the supernatant for test. **(Note that animal tissues and other samples with higher protein content are recommended to be centrifuge for 20-30 minutes or repeated for 2-3 times until the supernatant is clarified).**

2. Bacteria or cells

According to the ratio of bacteria or cells(10^4) and extract solution volume(mL) is 500~1000:1 to add the extract solution (It is recommended to add 0.6 mL of extract reagent to 5 million of bacteria or cells, then extract with ultrasonic cell disruption extraction method (placed on ice, ultrasonic power 200 W, working time 3 seconds, interval 7 seconds, total time 3 min). After extraction, add 0.4 mL of distilled water and centrifuge at 13000 ×g for 10 minutes at 25℃ to remove insoluble materials and take the supernatant for test.

3. **Liquid:** detect directly.

II. Determination

1. Preheat microplate reader/spectrophotometer for 30 minutes, adjust wavelength to 704 nm, set zero with distilled water.

2. Standard: Dilute the 10 mg/mL(10000 μg/mL) standard solution to 250 、 125 、 62.5 、 31.25 、 15.625、 7.8125μg/mL with Diluent solution.

3. Perform the following operations in 1.5 mL EP tubes.

Reagent (μL)	Test tube (T)	Standard tube (S)	Blank tube （B）
Sample	25	-	-
Diluent solution	-	-	25
Standard solution	-	25	-
Reagent I	20	20	20
Reagent II	25	25	25
Mix well and incubate the reaction for 30 min at 80°C.			
Reagent III	20	20	20
Reagent IV	55	55	55
Reagent V	30	30	30
Distilled water	75	75	75
Mix thoroughly, take 200 μL of liquid in micro glass cuvette or 96 well plate. Detect the absorbance value of each tube at 704 nm and noted as A_T, A_S, and A_B. $\Delta A_T=A_T-A_B$, $\Delta A_{S=}A_S-A_B$. Blank tubes only need to be tested 1-2 times.			

III. Calculation

1. Standard curve.

The concentration of standard solution as x-axis, ΔA_B as y-axis, obtain the equation y=kx+b. Take ΔA_T to the equation to acquire x value.

2. Calculate

1. Protein concentration

VB1 (μg/mg prot)=$x\times V_{ST}\div(V_{ST}\times Cpr)$ = $x\div Cpr$

2. Sample weight

VB1 (μg/g)=$x\times V_{ST}\div W= x\div W$

3. Bacterias or cells

VB1 (μg/10^4 cell)=$x\times V_{ST}\div Nc= x\div Nc$

4. Liquid sample

VB1 (μg/mL)=x×V_{ST}÷V_{ST}= x

V_{ST} : Extract solution volume, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

Nc: The total number of bacteria or cells, 10^4 .

Note:

1. If A>1.2, the sample can be determined after being appropriately diluted with Diluent solution. When calculation, multiply the calculation formula by the corresponding dilution factor.

2. Samples with higher protein concentrations, such as animal tissues, legume seeds and so on are recommended to dilute the sample 20 or 40 times before determing, multiply the calculation formula by the corresponding dilution factor.

3. Absorbances are measured immediately after color development is completed and try to ensure the same reaction time. If there is precipitation after color rendering,shake it well and measure it.

Technical index:

1. The lowest detection limit: 0.057μg/mL.
2. Linearity range: 1.953-250 μg/mL.

Experimental Examples:

1. Take 0. 1g of kidney, add 0.6mL extract, extract at 60℃ for 30min, add 0.4mL of distilled water, mix well, centrifuge at 25℃, 13000rpm for 10min, take the supernatant, dilute the supernatant by 40 times and follow the determination procedure Operation, measured and calculated ΔA=At-Ab=0.551-0. 107=0.444, brought into the standard curve y=0.0054x+0.0043, calculated x=81.43μg/mL, calculated VB1 content according to the sample quality:

VB1 (μg/g mass) = x×V sample total ÷W×40 (dilution multiple)= 81.43×1÷0. 1×40=32572 μg/g mass.

2. Take 0. 1g of clover, add 0.6mL extract, extract at 60℃ for 30min, add 0.4mL of distilled water, mix well, centrifuge at 25℃, 13000rpm for 10min, take the supernatant, dilute the supernatant by 40 times and follow the determination procedure Operation, measured and calculated ΔA=At-Ab=0.727-0. 107=0.62, brought into the standard curve y=0.0054x+0.0043, calculated x =114.02 μg/mL, calculated VB1 content according to the sample quality:

VB1 (μg/g mass) = x×V sample total ÷W×40 (dilution multiple)= 114.02×1÷0. 1×40=45608 μg/g mass.

Related Products:

AK0406/AK0405 Vitamin B6(VB6) Content Assay Kit

AK0280/AK0279 Vitamin E(VE) Content Assay Kit