Vitamin B6 Content Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: NA0720

Size: 50T/24S

Components:

Extract solution**:** Liquid 18 mL×1. Store at 4℃.

Reagent I: Liquid 30 mL×1. Store at 4℃.

Reagent II: Liquid 15 mL×1. Store at 4℃.

Reagent III: Liquid 20 mL×1. Store at 4℃ and protect from light.

Reagent IV: Powder×1. Store at 4℃ and protect from light. Dissolve it with 20 mL of distilled water before use and the reagent can be stored for one week at -20℃.

Standard: Powder×1, 10 mg of Vitamin B6. Store at 4℃. Dissolve it with 1 mL of Reagent I to prepare as 10000 μg/mL standard solution before use.

Description:

Vitamin B6 (VB6), also known as pyridoxine, which includes pyridoxine, pyridoxal and pyridoxamine, is present in the form of phosphates in the body. VB6 is a water-soluble vitamin, which is plentiful in meat, whole grains, vegetables and nuts. VB6 participate in the metabolism of a variety of proteins and amino acids in the body, which has an extremely important role in the organism.

Under the action of strong oxidant, VB6 and 4-aminoantipyrine form a stable yellow compound which has characteristic absorption peak at 400 nm. In this kit, the content of VB6 is calculated by measuring the absorbance at 400 nm.

Required but not provided:

Spectrophotometer, centrifuge, transferpettor, water bath, 1 mL glass cuvette, mortar/homogenizer, EP tube and distilled water.

Protocol:

I. Sample preparation

1. Tissue

Crushing the tissue samples and according to the ratio of crushed 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 Extract solution to 0.1 g of crushed tissue sample), extract the reaction for 30 minutes at 60℃. After extraction, add 0.4 mL of distilled water and centrifuge at 16000 rpm 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 solution to 5 million of bacteria or cells, then extracting with ultrasonic cell disruption extraction method (placed on ice, ultrasonic power 300W, working time 3 seconds, interval 7 seconds, total time for 3 min). After extraction, add 0.4 mL of distilled water and centrifuge at 16000 rpm for 10 minutes at 25℃ to remove insoluble materials and take the supernatant for test.

3. Liquid: detect directly.

II. Detection

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 400 nm, set zero with distilled water.

2. Standard: Dilute the 10000 μg/mL standard solution to 1、0.5 、0.25 、0.125 、0.0625 、0.03125 、0.015625 mg/mL with Reagent I.

3. Add reagents as the following table.

Reagent (μL)	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube（B）
Sample	200	200	-	-
Standard	-	-	200	-
Reagent I	-	-		200
Reagent II	200	200	200	200
Reagent III	300	300	300	300
Reagent IV	300	-	300	300
Distilled water	-	300	-	-
Mix thoroughly and the mixture incubated at 25℃ for 20 minutes. Add the mixture into 1 mL glass cuvette, and detect the absorbance value of each tube at 400 nm and noted as A_T, A_C, A_S and A_B. $\Delta A_T=A_T-A_C$, $\Delta A_{S=}A_S-A_B$. Blank tube 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 (μg/mL) value.

2. **Calculate.**

1. Protein concentration

VB6 (mg/mg prot)=$x\times V_{ST}\div(V_{ST}\times Cpr)= x\div Cpr$

2. Sample weight

VB6 (mg/g weight)=$x\times V_{ST}\div W= x\div W$

3. Bacterias or cells

VB6 (mg/10^4 cell)=$x\times V_{ST}\div Nc= x\div Nc$

4. Liquid sample

VB6 (mg/mL)=$x\times V_{ST}\div V_{ST}= x$

V_{ST}: Extract solution volume, 1 mL;
Cpr: Sample protein concentration, mg/mL;
W: Sample weight, g;
V_{ST}: Sample volume, 0.2 mL;
Nc: The total number of bacteria or cells, 10^4.

Note:

1. If A>0.8, the sample can be determined after being appropriately diluted with Reagent I. When calculation, multiply the calculation formula by the corresponding dilution factor.
2. Samples with higher protein concentrations, such as animal tissues, legume seeds, etc., if precipitation occurs after color development is completed, the sample is diluted and then measured, 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.

Experimental examples:

1. Weigh 0.1g of mouse muscle tissue, add 0.6 mL of Extract solution, extract at 60℃ for 30 min, add 0.4 mL of distilled water, mix well, centrifuge at 25℃ and 16000 rpm for 10 min, take the supernatant, and then operate according to the determination steps, calculate $\Delta A = A_T - A_C = 0.1916 - 0.1238 = 0.0678$, standard curve y=1.1434x-0.009, calculate x = 0.0672, calculate VB6 content according to sample mass.
VB6 (mg/g mass) = $x \times V_E \div W = 0.0672 \times 1 \div 0.1 = 0.672$ mg/g mass.
2. Weigh 0.1 g of peanut, add 0.6 mL of Extract solution, extract at 60℃ for 30min, add 0.4 mL of distilled water, mix well, centrifuge at 25℃ and 16000rpm for 10 min, take the supernatant, then operate according to the determination steps, use 96 well plate to measure and calculate $\Delta A = A_T - A_C = 0.4473 - 0.2885 = 0.1588$, standard curve y=1.1434x-0.009, calculate x=0.147.
VB6 (mg/g mass) = $x \times V_E \div W = 0.147 \times 1 \div 0.1 = 1.47$ mg/g mass.

Related Products：

NA0335/NA0334 Vitamin B1(VB1) Content Assay Kit
NA0757/NA0515 Vitamin E(VE) Content Assay Kit

Technical Specifications:

The detection limit: 0.0162 mg/mL
The linear range: 0.015625-1 mg/mL