

Vitamin B1 (VB1) Content Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: NA0335

Size: 50T/48S

Components:

Extract solution: Liquid 35 mL×1. Store at 4°C. Contains insoluble matter, shake well before use.

Diluent solution: Liquid 60 mL×1. Store at 4°C.

Reagent I: Liquid 6 mL×1. Store at 4°C.

Reagent II: Liquid 7 mL×1. Store at 4°C and protect from light.

Reagent III: Liquid 6 mL×1. Store at 4°C.

Reagent IV: Liquid 15 mL×1. Store at 4°C.

Reagent V: Liquid 8 mL×1. Store at 4°C and protect from light.

Standard: Powder×1, 10 mg Vitamin B1. Store at 4°C **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, centrifuge, transferpettor, water bath, 1 mL glass cuvette, 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°C. After extraction, add 0.4 mL of distilled water and centrifuge at 13000×g for 10 minutes at 25°C 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 $13000 \times g$ for 10 minutes at 25°C 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 704 nm, set zero with distilled water.
2. Standard: Dilute the 10mg/mL(10000 $\mu\text{g}/\text{mL}$) standard solution to 250、125、62.5、31.25、15.625、7.8125 $\mu\text{g}/\text{mL}$ 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	100	-	-
Diluent solution	-	-	100
Standard solution	-	100	-
Reagent I	80	80	80
Reagent II	100	100	100
Mix well and incubate the reaction for 30 min at 80°C .			
Reagent III	80	80	80
Reagent IV	220	220	220
Reagent V	120	120	120
Distilled water	300	300	300
Mix thoroughly, add the mixture into 1 mL glass cuvette . 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

$$VB1 (\mu\text{g}/\text{mg prot}) = x \times V_{ST} \div (V_{ST} \times C_{pr}) = x \div C_{pr}$$

2. Sample weight

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

3. Bacterias or cells

$$VB1 (\mu\text{g}/10^4 \text{ cell}) = x \times V_{ST} \div N_c = x \div N_c$$

4. Liquid sample

$$VB1 (\mu\text{g/mL}) = x \times V_{ST} \div V_{ST} = x$$

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

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

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 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 determination, 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: 7.366 $\mu\text{g/mL}$.
2. Linearity range: 7.8125-250 $\mu\text{g/mL}$.

Experimental Examples:

1. Take 0.1g of kidney, add 0.6mL extract, extract at 60°C for 30min, add 0.4mL of distilled water, mix well, centrifuge at 25°C, 13000rpm for 10min, take the supernatant, dilute the supernatant by 20 times and follow the determination procedure Operation, measured and calculated $\Delta A = A_t - A_b = 0.339 - 0.265 = 0.074$, brought into the standard curve $y = 0.006x + 0.001$, calculated $x = 12.17 \mu\text{g/mL}$, calculated VB1 content according to the sample quality:

$$VB1 (\mu\text{g/g mass}) = x \times V_{\text{sample total}} \div W \times 20 (\text{dilution multiple}) = 12.17 \times 1 \div 0.1 \times 20 = 2434 \mu\text{g/g mass.}$$

2. Take 0.1g of clover, add 0.6mL extract, extract at 60°C for 30min, add 0.4mL of distilled water, mix well, centrifuge at 25°C, 13000rpm for 10min, take the supernatant, dilute the supernatant by 40 times and follow the determination procedure Operation, measured and calculated $\Delta A = A_t - A_b = 0.748 - 0.265 = 0.483$, brought into the standard curve $y = 0.006x + 0.001$, calculated $x = 80.33 \mu\text{g/mL}$, calculated VB1 content according to the sample quality:

$$VB1 (\mu\text{g/g mass}) = x \times V_{\text{sample total}} \div W \times 40 (\text{dilution multiple}) = 80.33 \times 1 \div 0.1 \times 40 = 32132 \mu\text{g/g mass.}$$

Related Products:

NA0720/NA0478 Vitamin B6(VB6) Content Assay Kit

NA0757/NA0515 Vitamin E(VE) Content Assay Kit