VB1 Assay Kit (Spectrophotometry)

Description

Vitamin B1 (VB1) is a major component of the decarboxylation coenzyme and participates in the tricarboxylic acid (TCA) cycle during cellular metabolism. It is a water-soluble vitamin essential for maintaining normal metabolic functions of the body and plays a crucial role in energy metabolism in living organisms.

Detection Principle

Vitamin B1 reduces potassium ferricyanide to potassium ferrocyanide under alkaline conditions. The resulting potassium ferrocyanide reacts with Fe^{3+} under weakly acidic conditions to form Prussian blue, which exhibits a characteristic absorption peak at 704 nm.

Packing

Taking 50T/48S for example:

Components	Packing	Storage
CB0190S-ES	40 mL×1	4 ℃
CB0190S-A	8 mL×1	4 ℃
CB0190S-B	6 mL×1	4 ℃
CB0190S-C	8 mL×1	4 ℃, protected from light
CB0190S-D	16 mL×1	4 ℃
CB0190S-E	10 mL×1	4 ℃, protected from light
CB0190S-Standard	1 vial (powder) ×1	4 ℃ Before use, add 1 mL of Reagent A to prepare a standard solution with a concentration of 5 mg/mL.
Prior to the formal determination, a preliminary assay should be conducted using 2-3 samples with large expected differences.		

Instructions

1. Preparation of Lab Instruments

Visible spectrophotometer, 1 mL glass cuvette, constant-temperature water bath, balance, mortar, centrifuge, and distilled water.

2. Crude Enzyme Extract Preparation

1) Tissue samples:

Grind the sample thoroughly. Add extraction buffer at a ratio of tissue weight (g) to extraction volume (mL) of 1:5–10 (recommended: weigh ~0.1 g tissue and add 0.6 mL CB0190S-A). Incubate at 60 °C for 30 min, then add 0.4 mL distilled water and mix well. Centrifuge at 13,000 × g, 25 °C for 10 min, and collect the supernatant for analysis.

For samples with high protein content (e.g., animal tissues), centrifugation for 20–30 min is recommended.

2) Cell samples:

Add extraction buffer at a ratio of 500–1000 cells (×10⁴) per 1 mL extraction buffer (recommended: 5 × 10^6 cells + 0.6 mL CB0190S-A). Lyse cells by ultrasonic disruption on ice (power 300 W; sonication 3 s on / 7 s off, total time 3 min). Add 0.4 mL distilled water, mix well, then centrifuge at 13,000 × g, 25 °C for 10 min. Collect the supernatant for analysis.

Serum and other liquid samples: Measure directly.

3. Measurement Procedure

Preheat the spectrophotometer for 30 min, set the wavelength to 704 nm, and zero the instrument using distilled water.

Add the following reagents sequentially into an EP tube:

	Blank Tube（μL）	Sample Tube（μL）	Standard Tube（μL）
Sample		100	
CB0190S-A	100		
CB0190S-B	80	80	80
CB0190S-C	100	100	100
Standard Solution			100
Thoroughly mix and incubate at 80 °C for 10 min.			
CB0190S-ES	80	80	80
CB0190S-D	220	220	220
CB0190S-E	120	120	120
H2O	300	300	300
Mix thoroughly and allow the mixture to stand for 20 min. Transfer to a 1 mL glass cuvette, use distilled water as the blank (zero adjustment), and measure the absorbance at 704 nm. Record the absorbance values as A blank, A sample, and A standard. Only 1–2 blank tubes are required.			

4. Calculation of VB1

Standard curve: **x-axis:** The concentrations of the standard solutions

y-axis (with A blank as the zero point of the standard): The corresponding A standard

y = kx + b. A sample is then substituted into the equation to calculate x (mg/mL).

1) Based on protein concentration

VB1 content (mg/mg prot) = x×V2÷（V2×Cpr）= x÷Cpr

2) Based on sample mass

VB1 content (mg/g) = x×V2÷W=0.6x÷W

3) Based on cell number

VB1 content (mg/104 cell) = x×V2÷cell number（×10,000）=0.6x÷cell number（×10,000）

4) Based on solution volume

VB1 content (mg/mL) = x×V1÷V1= x

Note: V1: Volume of sample added, 0.1 mL; V2: Volume of the sample extraction, 0.6 mL;

Cpr: Protein concentration, mg/mL; W: Sample weight, g

Precautions

1. For protein quantification, it is recommended to use BCA Protein Quantification Kit (C0050).
2. If the absorbance value exceeds 1, dilute the sample before measurement and multiply the calculated result by the dilution factor.
3. For samples with high protein concentrations, such as animal tissues, if precipitation occurs after color development, dilute the sample and re-measure, and multiply the result by the dilution factor.
4. Measure the absorbance immediately after color development is complete.
5. The linear range of the standard curve is 0.1–10 μg/mL.
6. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
7. Please wear a lab coat and disposable gloves.