VB6 Assay Kit (Spectrophotometry)

Description

Vitamin B6 (VB6), also known as pyridoxine, includes pyridoxine, pyridoxal, and pyridoxamine. In the body, it exists mainly in the form of phosphate esters and is a water-soluble vitamin. Vitamin B6 participates in a wide range of protein and amino acid metabolic processes within cells and plays an extremely important role in biological systems.

Detection Principle

VB6 reacts with 4-aminoantipyrine in the presence of a strong oxidizing agent to form a stable yellow compound, which exhibits a characteristic absorption peak at 400 nm.

Packing

Taking 50T/48S for example:

Components	Packing	Storage
CB0308S-ES	35mL×1	4 ℃
CB0308S-A	8mL×1	4 ℃
CB0308S-B	15mL×1	4 ℃
CB0308S-C	20mL×1	4 ℃, protect from light
CB0308S-D	20mL×1	4 ℃, protect from light
CB0308S-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 CB0308S-ES). 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 × 10⁶ cells + 0.6 mL CB0308S-ES). 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

1. Set the visible spectrophotometer to a wavelength of 400 nm and zero the instrument using distilled water.
2. Dilute the 5 mg/mL standard solution with Reagent A to prepare standard solutions at 250, 125, 62.5, 31.25, 15.625, and 7.8 μg/mL.
3. Add the following reagents sequentially into EP tubes:

	Blank Tube（μL）	Sample Tube（μL）	Standard Tube（μL）
CB0308S-A	200		
Sample		200	
Standard Solution			200
CB0308S-B	200	200	200
CB0308S-C	300	300	300
CB0308S-D	300	300	300
Mix thoroughly and allow the mixture to stand for 30 min (until the solution turns yellow). Transfer to a 1 mL glass cuvette and measure the absorbance at 400 nm, recorded as A blank, A standard, and A sample. ΔA standard = A standard − A blank. Only 1–2 blank tubes are required.			

4. Calculation of VB1

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

y-axis: The corresponding ΔA standard

y = kx + b. ΔA is then substituted into the equation to calculate x (μg/mL).

1) Based on protein concentration

VB6 content (μg/mg prot) = x×V2÷（V2×Cpr）= x÷Cpr

2) Based on sample mass

VB6 content (μg/g) = x×V2÷W=0.6x÷W

3) Based on cell number

VB6 content (μg/10^4 cell) = x×V2÷cell number（×10,000）=0.6x÷cell number（×10,000）

4) Based on solution volume

VB6 content (μg/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 the linear range, 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 product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
6. Please wear a lab coat and disposable gloves.