

Vitamin B₁₂ Kit(ready-to-use)(GB)

(Product No. GVT1002)

GFAD[02]1.23

1. Introduction

This product is an ready-to-use kit for Vitamin B₁₂ detection by tubes method, developed in accordance with the standard “GB5009.285-2022” , each product box contains 2 sets of reagents and each reagent preparation in tubes (50) .

2. Principle of the Method

Vitamin B₁₂ is an essential nutrient for the growth of *Lactobacillus leichmannii* (ATCC 7830). Under certain controlled conditions, *Lactobacillus leichmannii* suspension is inoculated into a culture medium containing the sample solution. After a period of incubation, the transmittance (or absorbance) is measured. The Vitamin B₁₂ content in the sample is then calculated based on the standard curve of Vitamin B₁₂ content versus transmittance (or absorbance).

3. Reagents provided

Vitamin B ₁₂ Standards (Freeze-dried)	2 vials
Vitamin B ₁₂ Bacterial Ball (Freeze-dried)	2 vials
Vitamin B ₁₂ Medium Base	250 mL×2
Vitamin B ₁₂ Medium Additive 1(Freeze-dried)	2 vials
Vitamin B ₁₂ Medium Additive 2(Freeze-dried)	2 vials

4. Storage condition:

Store in dark place 2-8°C for a year.

5. Materials required but not provided

5.1 Ultra-clean workstation	5.7 Sterile tubes and racks
5.2 Constant temperature incubator, 37°C ±1°C	5.8 Pipette and sterile tips, 10-100 μL, 100-1000 μL, 500-5000μL
5.3 Uv-vis spectrophotometer	5.9 Sterile water
5.4 Autoclave	5.10 Sterile centrifugal tubes with cover: 15 mL,50 mL
5.5 Ultrasonic oscillator	5.11 Sterile syringes and 0.22 μm sterile filter membrane
5.6 Vortex mixer	

6. Assay medium preparation (aseptic procedure)

6.1 Preparation of Vitamin B₁₂ Assay Medium

6.1.1 **Vitamin B₁₂ Medium Additive1** and **Vitamin B₁₂ Medium Additive2**, add 5mL **Vitamin B₁₂ Medium Base** to each one. Mix for 3-5 minutes, complete mixing, and then add all of **Vitamin B₁₂ Medium Additive1,2** into 250mL **Vitamin B₁₂ Medium Base**, and mix well.

6.1.2 Take 1 vial of **Vitamin B₁₂ Bacterial Ball** add into (6.1.1)the assay medium,mix well.

6.2 Preparation of standard solution

6.2.1 Vitamin B₁₂ standard solution: Add 5ml sterile water to **Vitamin B₁₂ Standard**, dissolve and mix well.

6.2.2 Vitamin B₁₂ standard working solution: Accurately take 2mL of Vitamin B₁₂ Standard solution(6.2.1) into 8ml of sterile water, mix and use.(Ready-to-use)

7. Preparation of Samples

According to the standard for sample processing and dilution.

8. Preparation of standard tubes (aseptic operation)

8.1 Standard tubes

Add sterile water, Vitamin B₁₂ working solution (6.2.2) , and Vitamin B₁₂ medium(6.1.2) to sterile test tubes according to Table 1.

Table 1-1 Preparation of standard curves

Number	S1	S2	S3	S4	S5	S6	S7	S8	S9
Sterile Water /mL	5.0	4.95	4.90	4.85	4.80	4.75	4.70	4.60	4.50
Vitamin B ₁₂ Working Solution /mL	0.0	0.05	0.10	0.15	0.20	0.25	0.30	0.40	0.50
Medium/mL	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00

The standard tubes,2-3 sets should be prepared.

8.2 Assay tubes

The prepared sample diluent was filtered by g0.22μm sterile filter membrane,and then add sterile water, sterile sample solution and medium(6.1.2.) in sterile test tube according to Table 2. Mix well.

Table 2: Preparation of assay tubes

Sample tube number	S1	S2	S3	S4
Sterile Water /mL	4.00	3.00	2.00	1.00
Sample solution /mL	1.00	2.00	3.00	4.00

Medium/mL	5.00	5.00	5.00	5.00
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9. Incubation

Incubate at 37°C ± 1°C for 44-48 h in an incubator, avoiding light. Be sure to grow the end.

10. Measurement

The cultured standard tubes, the assay tubes and the enzyme blank tubes were used a vortex shaker, mix well. A microplate reader or a cuvette with thickness 1cm was used for determination at 550nm.

The absorbance outside the range of standard curve tube S1-S9 was discarded.

The concentration measured in each tube must not exceed 15% of this average value, and any values exceeding this limit should be discarded. If the number of tubes meeting this requirement is less than 2/3 of the total number of tubes for all four numbered test solutions, the data used to calculate the sample content is insufficient and retesting is required. If the number of tubes meeting the requirement exceeds 2/3 of the original number, recalculate the average concentration of Vitamin B12 per milliliter in the valid test tubes for each number. Use this average to calculate the overall average value "C_x" for all numbered sample wells.

11. Data analysis

Analyze the results according to the national standard GB 5009.285-2022.

11.1 **Standard curve:** Using the Vitamin B₁₂ content of the standard series tubes as the abscissa and the transmittance (or absorbance value) of each standard point as the ordinate, a standard curve is plotted.

11.2 Result calculation:

From the standard curve to calculate samples concentration of vitamin B₁₂ in test tubes, and then according to the degree of dilution and calculation to the sample weight VB₁₂ concentrations in the samples.

the results are calculated according to formulas (1) :

$$X = \frac{C_x}{m} \times \frac{f}{1000} \times 100 \quad \dots\dots(1)$$

X— Vitamin B₁₂ content in the sample, ng/mL;

C_x—(11.2) averages, ng;

m—sample mass g;

f— Dilution factor of sample extract;

Under repeated conditions, the arithmetic mean value of two independent measurement results is obtained, and the value retains three significant digits.

For laboratory use in industry or R&D purpose. Not for drug, household or other uses.

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