Vitamin B_{12} Kit（Ready to use）

（Product No. GVT2002 ）

GFAD[01]09.22

1. Introduction

This product is a ready-to-use kit for vitamin B_{12} detection by the microbiological assay technique .developed in accordance with the standard “GB5009.285-2022”， each product box contains 3 sets of reagents.

2. Principle of the Method

Vitamin B_{12} 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 B_{12} content in the sample is then calculated based on the standard curve of vitamin B_{12} content versus transmittance (or absorbance).

3. Reagents provided

Vitamin B_{12} Standards（Freeze-dried）	3 vials
Vitamin B_{12} Bacterial Ball（Freeze-dried）	3 vials
Vitamin B_{12} Medium Base (20 mL)	3 vials
Vitamin B_{12} Medium Additive (Freeze-dried)	3 vials
Sterile Water(30mL)	3 vials
Sterile 96-well Microplate individually packed	3 plates
Sealing Film	3 pieces

4. Storage condition:

Store in dark place 2-8℃ for a year

5. Materials required but not provided

5.1 Ultra-clean workstation

5.2 Constant temperature incubator, 36℃ ±1℃

5.3 Microplate reader (540nm~610 nm)

5.4 Autoclave

5.5 Ultrasonic oscillator

5.6 Vortex mixer

5.7 Pipette and sterile tips, 10-100 μL, 100-1000 μL

5.8 conical flask and volumetric flask

5.9 Pipette centrifuge tubes: 1.5 mL, 15 mL tubes should have screw tops

5.10 Sterile syringes and 0.22 μm sterile filter

membrane

6. Assay medium preparation (aseptic procedure)

6.1 Preparation of Vitamin B_{12} Assay Medium: Pipette 1.0mL of the basic vitamin B_{12} assay medium into the additive of the Vitamin B_{12} Assay Medium, dissolve for 3 minutes to ensure complete mixing, and then transfer the entire mixture into 20mL of the basic vitamin B_{12} assay medium, and mix well.

6.2 Non-inoculation of standard 0 control tube medium: 500 μL of the aforementioned medium was taken and placed in a 1.5mL sterile centrifuge tube, which serves as the medium for the non-inoculated standard 0 control tube as S1.

6.3 Inoculation of Vitamin B_{12} Assay Medium: take 1 vial of of vitamin B_{12} bacterial ball, add it to the prepared Vitamin B_{12} Assay Medium (6.1), mix well and then use.

7. Preparation of standard tubes (aseptic operation)

This product provides the following two methods for preparing standard series tubes. "Method 1" is consistent with the national standard procedure, while "Method 2" omits the step of diluting the standard working solution. Both methods yield standard series tubes with identical vitamin B_{12} content, and you can choose either method depending on the situation.

Method 1: Accurately pipette 1.1 mL of sterile water into the Vitamin B_{12} Standard, dissolve and mix thoroughly. Then take 1 mL of the solution and add it to 4 mL of sterile water, mix well, and this is the Vitamin B_{12} Standard working solution. Take 10 sterile 1.5 mL centrifuge tubes and prepare a series of standard solutions with varying concentrations according to Table 1-1.

Table 1-1 Preparation of standard curves

Number	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Vitamin B_{12} content /ng	0.00	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.10
Standard solution /μL	0	0	100	200	300	400	500	600	800	1000
Sterile Water /μL	1000	1000	900	800	700	600	500	400	200	0

Method 2: Accurately pipette 1.1 mL of sterile water into the Vitamin B_{12} Standard, dissolve and mix thoroughly. Then take 10 sterile 1.5 mL centrifuge tubes and prepare a series of standard solutions with varying concentrations according to Table 1-2.

Table 1-2 Preparation of standard curves

Number	S1	S2		S3	S4	S5	S6	S7	S8	S9	S10
Vitamin B_{12} content /ng	0.00	0.00		0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.10
Standard solution /μL	0	0		20	40	60	80	100	120	160	200
Sterile Water /μL	1000	990		980	960	940	920	900	880	840	800

8. Preparation of Sample Tubes

8.1 The sample is prepared in accordance with step "19.2" in GB 5009.285-2022. "Third Method Microbial Method".

8.2 The prepared sample diluent is filtered and sterilized under sterile conditions using a sterile aqueous phase filter membrane (0.22μm),and a series of sample tubes are prepared in the order specified in Table 2.

Table 2: Preparation of specimen tubes

Sample tube number	1	2	3	4
Sample solution /μL	100	200	300	400
Sterile Water /μL	400	300	200	100

9. Detection steps (aseptic operation)

9.1Take out the sterile 96-well microplate, record the well positions, and conduct a parallel test in triplicate for each gradient of the standard solution as well as the sample diluent.

9.2 Add 150 μL of prepared inoculated Vitamin B_{12} Assay Medium (6.3) to each well, with the exception of the uninoculated standard control tube S1, which should receive 150 μL of uninoculated Vitamin B_{12} Assay Medium (6.2).

9.3 Transfer 150 μL of the standard series tube (7) or sample series tube (8.2) into the designated well.

9.4 Seal the wells on the strip with a sealing film and press the film to ensure that all wells are adequately sealed (The edge part of the wells section should be fully sealed, with special attention paid to it)

10. Incubation

Incubate at 36°C ± 1°C for 40-48 h in an incubator, avoiding light.

11.Measurement

11.1 Take out the plate from the incubator and press the sealing film again to ensure that all wells are adequately sealed. Shake the plate upside down repeatedly to mix the microorganisms well.

11.2 Remove the sealing film diagonally and puncture the air bubbles on the surface of each well with a needle.

11.3 Measure the absorbance value at 550 nm with a microplate reader.

If the 0 control well appears turbid, it indicates possible contamination by miscellaneous bacteria, and the test needs to be redone.

12. Data analysis

Analyze the results according to 'Part 20: Expression of Analytical Results' in the national standard GB 5009.285-2022.

12.1 Standard curve: Using the vitamin B_{12} concentration of the standard series tubes as the abscissa and the average transmittance (or absorbance value) of each standard point as the ordinate, a standard curve is plotted.

12.2 Result calculation:

The concentration of Vitamin B_{12} in the test solution is calculated from the standard working curve based on its light transmittance (or absorbance). Subsequently, the content of Vitamin B_{12} in the sample is determined using the dilution factor and the weighed sample quantity. Test values with transmittance (or absorbance) falling outside the range of S3 to S10 of the standard curve tubes should be discarded.

Calculate the concentration of Vitamin B_{12} per milliliter in each numbered test solution using the light transmittance (or absorbance) of each well. Then, calculate the average concentration of Vitamin B_{12} in the test solution of that number. The concentration measured in each well must not exceed 15% of this average value, and any values exceeding this limit should be discarded. If the number of wells meeting this requirement is less than 2/3 of the total number of wells for all four numbered test solutions, the data used to calculate the sample content is insufficient and retesting is required. If the number of wells meeting the requirement exceeds 2/3 of the original number, recalculate the average concentration of Vitamin B_{12} per milliliter in the valid test tubes for each number. Use this average to calculate the overall average value "p" for all numbered sample wells.

Calculate the content of Vitamin B_{12} in the sample, denoted as X, using Formula (1):

The content of Vitamin B12 (calculated as cyanocobalamin) in the sample is calculated using Formula (1)

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

X—Vitamin B_{12} content in the sample diluent,μg/100 g(mL);

ρ—The overall average concentration of Vitamin B12 in the valid test tubes, expressed in units of ng/mL;

f— Dilution factor of sample extract;

100—Unit conversion coefficient

m —sample mass g;

1000—Unit conversion coefficient

12.3 Description

The result of the calculation retains three significant figures.
Note: The Vitamin B_{12} content of liquid samples can also be measured in micrograms per 100 milliliters (μg/100mL).
Refer to national standards for precision, detection limit, and quantification limit.

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

Beijing Landbridge Technology co.,LTD
Add.：No. 3, North Gaobeidian Road, Chaoyang District, Beijing（100123）
Shandong：Room 703, Block A, Heda Center, 177 Tailiu Road, North district, Qingdao（266033）
Guangdong：East 4th floor, building A, Jinhe Industrial Park,
Shibei industrial road, Panyu District, Guangzhou（511400）
Northeast：Room 808, National Milk Center, No. 2727, Innovation Road, science and Technology Innovation City, Songbei district, Harbin
Chengdu：Room 204, unit 1, building 1, Zhonghai International Orange County Phase I, high-tech West district, Chengdu, Sichuan Province（610096）
Shanghai：Room 406, floor 4, building B, no. 455, Yanzhan Road, Songjiang District Emerging Industrial Park, Caohejing, Shanghai
Hotline：010-51203999　　0532-82689263
020-38011430　　0451-87821139
Website：www.beijinglandbridge.com
E- mail：tech_e@beijinglandbridge.com