Vitamin B12 (Vitamin B12) Test Kit

VB12 Test

Product Number: R6003

Product Unit: 1 plate, 96T

FOR Professional and Laboratory use only

Manufacturer Information

Ring Biotechnology Co., Ltd

E-mail: export@ringbio.com diego@nbgen.com Web: www.ringbio.com

Add: Building 3, Zhongtongtai TechnoPark, No. 11, Kechuang 14th St, Beijing 100176, CHINA

Tel: +86-10-56267496 Technical Support & Service: +86-13811393460

1. Introduction

Vitamin B12, also called cobalamin, is a water-soluble vitamin that is involved in the metabolism of every cell of the human body. It is a cofactor in DNA synthesis, and in both fatty acid and amino acid metabolism. It is particularly important in the normal functioning of the nervous system via its role in the synthesis of myelin and in the maturation of developing red blood cells in the bone marrow.

2. Principle of the Test

The current vitamin B12 Test Kit is based on microbiological method for the quantitative determination of the total vitamin B12 in food, animal feed and pharmaceutical products. The kit principle is accordance with ISO standard. Vitamin B12 is extracted from sample and then diluted. The diluted sample extract and substrate are added into the microtiter plate well which is coated with Lactobacillus delbrueckii (ATCC 7830). Incubate in the dark at 37°C (98.6°F) for 44-48 hours. The bacteria grows until the vitamin is consumed. The metabolism and growth strength of the bacteria was related to the turbidity of vitamin B12 extract. The measurement is done using an ELISA reader at 610-630 nm alternatively at 540-550 nm.

3. Kit Components

(1) Microtiter plate, 96wells, 1plate
(2) Sterile water 30ml, 3 bottles
(3) Assay Medium, 3 bottles
(4) VB12 Standard, 3 bottles
The final standard series is **0, 0.03, 0.06, 0.09, 0.12, 0.18 μg/ml**
(5) Microplate cover, 2pcs

4. Required reagents and instruments (not provided)

4.1 Reagent and Solution

(1) Sodium hydroxide NaOH, 2mol/L, 0.1mol/L, 1mol/L
2mol/L NaOH solution: 8g NaOH add in 100ml sterile water or deionized water.
(2) 1% NaCN or KCN, 100mg NaCN or KCN dissolved in 10ml sterile water.
(3) Hydrochloric acid, HCl, 1mol/L, 0.1mol/L
(4) Sterile water or deionized water
(5) Acetate buffer (pH 4.5, 0.05mol/L):
0.66g anhydrous sodium acetate dissolved in 50mL sterile water or deionized water, stir to dissolve, and then add 0.69ml acetic acid(>99%, AR), adjust to pH 4.5, use HCl when necessary. Then transfer the solution to another 100ml volumetric flask and make up to 100ml with deionized water or sterile water.
Note: The buffer can be stored for 1 week at 2-8°C.
(6) Amylase, Z.B fluke 86250

4.2 Instrument

(1) Aseptic bench
(2) ELISA reader 610-630nm (540-550nm)
(3) Incubator with dark chamber, 37°C (98.6oF)
(4) Water bath 95°C
(5) pH meter
(6) Centrifuge > 8000 x g
(7) Sterile pipette 20-200μL; 100-1000μL
(8) Sterile centrifuge vials with screw cap 15ml and 50ml
(9) Sterile vials 1.5-2.0 ml
(10) Spiral glass pot 500ml
(11) Volumetric flask 100 and 1000ml
(12) Beaker 100ml
(13) Sterile filters polyethersulfone 0.2μm with sterile filter

5. Sample preparation

5.1 Notice

(1) To determine the added vitamin B12 in vitamin-enriched solid samples usually extract with hot water.
(2) To determine the added vitamin B12 in liquid samples usually requires sterile filtration and dilution with sterile water.
(3) To determine the total vitamin B12 in samples must be treated with NaCN/KCN and enzyme.
(4) Samples should be stored in the dark at 4°C.
(5) Standards and samples should be in **triplicate**.
(6) Unknown Samples extract should be diluted twice.
(7) The sample extract should be prepared freshly before analysis and should be stored in dark place.

5.2 Sample extraction

(1) Add 1 g of homogenized sample to 40 mL of sterile water or deionized water or extraction solution at a dilution factor of 40. The dilution factor is directly included in the standard curve.
(2) Samples with low vitamin B12 concentration, the sample volume should be increased by 5g (mL) (calculation should be taken into consideration).
The following samples must **be sterile filtered** or **aseptically extracted**:

1) Samples not heated at the time of extraction, such as juice or healthy drinks (excluding samples heated at 95°C in a water bath for 30 min).
2) Contains Chinese medicine and seasoning samples as well as honey and tea.
3) Vitamin mixtures, premixes or tablets (samples with higher vitamin B12 content) (except samples heated for 30 min in 95°C water bath).
4) Darker samples with lower vitamin levels (filter first to remove the color).
5) If the sample contains solid particles or the sample haze affects filtration, it should be sterile filtration before centrifugation for 5 min (> 8000 x g).

Note: The sample do not need sterile filtration if the sample heated at 95°C for 30 min.
Dilution must be carried out with the sterile water provided in the kit. (The medium must be filtered)

5.3 Dilution (IMPORTANT)

(1) Dilution factor

Example: If a test sample with concentration of 1.2μg / 100g. Divide this concentration by the concentration of standard 2 to obtain the dilution factor.

Dilution factor = 1.2μg/0.06μg = 20

So the dilution factor should be 20, sample should be dilute at 1:20.

Dilution step

1) 1:10 (100μl sample extraction + 900μl sterile water from the test kit). **(Solution A)**

2) 1:2 (500μl **Solution A** + 500μl sterile water from the test kit)

Note: shake gently after each dilution to mix thoroughly.

5.4 Sample preparation Protocols

5.4.1 Liquid sample (multivitamin-containing juices and healthy drinks)
Add 1 mL sample to a 50 mL sterile centrifuge vial, fill up to 40 mL with sterile water or deionized water, mix, sterile filter (or heat the sample in 95°C water bath for 30 min, chill down quickly to below 30°C). Then transfer some of the extract to 1.5 or 2.0mL sterile reaction vial, depending on the concentration range, further dilutions with sterile water are necessary.

5.4.2 Pectin and candy sample

(1) Weight 15-20g pectin sugar or candy, into a 50 mL sterile centrifuge vial, fill up to 40 mL with sterile water or deionized water, dissolve the sample in 95°C water bath, chill down quickly to below 30°C.
(2) The extract solution was quantitatively transferred to a 100 mL volumetric flask with sterile or deionized water. Transfer the extraction solution containing approximately **1g sample** into a 50 mL sterile centrifuge vial, fill up to 40 mL with sterile water or deionized water, mix, sterile filter (or

heat the sample in 95°C water bath for 30 min, chill down quickly to below 30°C). Then transfer some of the extract to 1.5 or 2.0 mL sterile reaction vial, depending on the concentration range, further dilutions with sterile water are necessary.
For example: If the sample is 17g pectin sugar, the solution transferred to the sterile centrifuge vial (extract solution containing 1g sample) is : 100mL/17g = 5.88mL/g.

5.4.3 Capsules, Pills, and Vitamin Mixtures

Before testing:

Calculate the weight of each capsule or pill (weigh 5 capsules or pills and average them), then pulverize the pills in a mortar or mixer (capsules can be extracted directly after cutting).

- 1g Sample preparation

(1) Weigh 1g pill, vitamin mixture or cut capsule, into a 500mL spiral glass pot, fill up to 400 mL with sterile water or deionized water, then add 500ul **NaCN(1%, prepared freshly before testing)** mix thoroughly.
(2) Heat the sample in 95°C water bath for 30 min, mix at least 5 times during the period, chill down quickly to below 30°C. The extract solution was quantitatively transferred to a 1000 mL volumetric flask with sterile or deionized water.
(3) Transfer 1mL the extract to a 50 mL sterile centrifuge vial, fill up to 40 mL with sterile water or deionized water, mix, sterile filter (or heat the sample in 95°C water bath for 30 min, chill down quickly to below 30°C).
Further dilute with sterile water in 1.5 or 2.0 mL sterile reaction vial if vitamin B12 concentration is not within the standard curve range.
Note: Pre-dilution factor of 1000 should be considered when calculating the results, and 1mL to 40mL in the dilution step is included in the standard curve.

- 0.2g Sample preparation

(1) Weigh 0.2g pill, vitamin mixture or cut capsule, into a 50mL spiral glass pot, add 20ml deionized water or sterile water, 500ul **NaCN(1%, prepared freshly before testing)** , shake gently to mix. Then fill up to 40 mL with **sterile water or deionized water, and** mix again.
(2) Heat the sample in 95°C water bath for 30 min, mix at least 5 times during the period, chill down quickly to below 30°C.
(3) Centrifuge at 8000g minimum for 5min. Then take the supernate into 1.5 or 2ml tube for further analysis. Further dilution will be required if vitamin B12 concentration is not within the standard curve range.

5.4.4 Cereals, baby food, bread, flour and dairy products

(1) Add 1.0g homogenized sample, into a 50 mL sterile centrifuge vial, fill up to 40 mL with **sterile water**, shake gently to mix. Heat the sample in 95°C water bath for 30 min, mix at least 5 times during the period, chill down quickly to below 30°C.
(2)The sample was further filtered using a 0.22 micron filter into 1.5 or 2.0 mL sterile reaction vial. According to the concentration range of vitamin B12, the filtrate may be further diluted with sterile water. Make sure the final diluted samples is within the standard solution range.

5.4.5 Determination of total vitamin B12

Note: the sample must be extracted with NaCN or KCN and enzyme.
(1) Weight 1g(mL) sample into a 50 mL sterile centrifuge vial, add 20ml sterile water or deionized water, 250ul **NaCN(1%, prepared freshly before testing), shake gently and then adjust the pH to 4.5 with HCl.**
Note: Use 20ml **acetate buffer (pH4.5)** instead of the 20ml sterile water if you wish not to adjust the pH. Then add 250ul **NaCN as mentioned.**
(2) Add 300mg amylase, shake gently and then incubate at 37°C for 1hour, shake time to time during the incubation. Make up the volume to 40ml with sterile water or deionized water.
(3) Heat the sample in 95°C water bath for 30 min, mix at least 5 times during the period, chill down quickly to below 30°C.
(3) Centrifugation at more than 8000xg for 5 min. According to the concentration range of vitamin B12, the supernate was further diluted with sterile water in a 1.5 or 2.0 mL sterile reaction vial.
Note: Further dilution will be required if vitamin B12 concentration is not within the standard curve range.

6. Assay Protocol

6.1 Preparing vitamin B12 standard solutions

(1) Open the sterile water bottle, pill up the blue lid through the edge of the glass and remove the whole bottle cap. Open the vitamin B12 standard bottle; place the inside of the bottle cap upward.

(2) Add sterile water into the standard bottle and close the cap. Shake and dissolve the standards fully and get the standard concentrate. The volume of sterile water added is specified on be

(3)Take 6 sterile vials (1.5-2.0ml), dilute the standard concentrate according to the following table. Standards have to be prepared freshly before the test.

Standard curve in µg/100g(ml)	Sterile water in µL		Standard concentrate in µl		Total volume in µl
Blank: 0	900	+	0	=	900
Standard 1: 0.03	900	+	100	=	1000
Standard 2: 0.06	400	+	100	=	500
Standard 3: 0.09	350	+	150	=	500
Standard 4: 0.12	300	+	200	=	500
Standard 5: 0.18	200	+	300	=	500

1:40 dilution factor is already included in the standard curve.

6.2 Preparing vitamin B12 assay-medium

(1) Open the bottle and discard the desiccant with tweezers.

(2) Add 10ml sterile water from the test kit to the vitamin B12 assay-medium, and then close the assay-medium bottle and shake gently.

(3) Heat the bottle in a water bath to 95°C for 5 min while shaking at least twice. Always make sure that the bottle is tightly closed, chill down quickly to below 30°C.

(4) Filter the medium through a 0.2µm filter into a sterile 15 ml centrifuge vial.

6.3 Assay procedure

Note: The sample added to the microplate must be sterile (Use the sterile water provided in the kit for dilution).

1) Take required microwell strips and keep the remaining microwell strips in a desiccant-containing aluminum foil bag and store at 2-8°C.
2) Add 150 µl vitamin B12 assay medium and 150 µl standard or sample into the wells (rinse tip with standard or sample solution), shake gently, and then cover the strips/cavities with plate cover.
   Note: make sure the plate is well covered to avoid contamination. Take special care with the wells around the plate edges.
3) Incubate at 37°C in the dark for 44-48 hours in an incubator.
4) Press down the plate cover once more, place the microtiter plate on a table and shake gently again to dissolve the microorganisms thoroughly in the medium.
5) Carefully pull off the plate cove.
6) Eliminate any bubbles on the surface of liquid in the wells with a pipette tip.
7) Measure the turbidity with an ELISA reader at 610 - 630 nm (alternatively at 540 - 550 nm).

Note: After incubation, the microtiter plate can be stored for max 48 hours in the refrigerator, and after then the plate shall be measured. To avoid any time losses due to weekends or holidays, schedule your experiment carefully.

7. Result determination

Recommend use a four points software (4 parameter logistic curve, 4PL) to calculate the results.

The test results are valid if the blank OD value is below to the OD value of standard 1, and the OD value of standard 5 is greater than 0.6.

$$\text{Vitamin B12 (µg/100 ml or µg/100 g)} = \frac{\text{Concentrate read from standard curve x } \textbf{dilution factor}}{\text{Amount of sample in ml (g)}}$$

Note: The default sample dilution factor 40 is already included in the standard curve.
The dilution factor in the formula is the dilution factor of the sample extract.
For example:
Sample quality; 1 g
Sample dilution: 1:40 (already in the curve, do not consider)
Sample extract dilution: 1:20 (must be consider)
Reading from the standard curve: 0.12µg/100g (ml)
The actual concentration in the sample is: 0.12×20/1=2.4 µg/100g (ml)
Note: remember to consider dilution factor if you have futher diluted the sample extraction.

8. Kit Performance

Time requirement:	Test setup in approx. 60 min Result calculation in 2 min
Incubation:	44-48hours in the dark at 37°C (98.6°F)
Standard Range:	0.03-0.18µg/100g (ml)
Recovery:	90-105%
Repeatability:	C.V. < 10 %

9. Notices

1) Kits should be stored at 2-8°C. Expired kits and reagent can not be used.
2) The sample extract or diluent added to the microplate must be sterile, and the sample must be diluted with sterile water provided in the box.
3) NaCN / KCN is toxic. Operate in a safe place or fume hood. Discard the waste carefully according to local regulations.
4) Other consumables needed in the experiment must also be sterile.
5) The assay-medium could evoke irritations of mucosa, eyes and skin.
6) After running the test, the microwell strips used must be disposed of according to regulations (e.g. autoclaved).

10. Storage and expiration

The kit is valid for 12 months when stored at 2-8°C. Production and expiry information are printed on the package.