VB12 (Cyanocobalamin) ELISA Kit

Catalog No: E-FS-M002

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

VB12 microtiter plate test is a microbiological method for the quantitative determination of VB12 microtiter plate test is a microbiological method for the quantitative determination of VB12 microtiter plate test is a microbiological method for the quantitative determination of VB12 in food products. The microbiological test system is in accordance with AOAC international norms.

VB12 is extracted from the sample and the extract is diluted. The VB12 assay-medium and the diluted extract are pipetted into the wells of a microtiter plate which is coated with Lactobacillus delbriickii. The growth of Lactobacillus delbriickii is dependent on the supply of VB12. Following the addition of VB12 as a standard or as a compound of the sample, the bacteria grow until the vitamin is consumed. The incubation is done in the dark at 37 ℃ (98.6 ℉) for 44 - 48 h.

The intensity of metabolism or growth of Lactobacillus delbriickii in relation to the extracted VB12 is measured as turbidity and compared to a standard curve. The measurement is done using a microtiter plate reader at 610 - 630 nm (alternatively at 540 - 550 nm).

Technical indicator

Reaction mode (Incubation time and temperature)**:** 37℃; 44-48 min.

Detection limit: 0.03μg/100g

Cross-reactivity: VB12 ---100%

Sample recovery rate: 100%±20%

Intra/Inter-assay CV for standards: <5 %

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
VB12 Standard (dissolved X mL)	3 bottles
VB12 Assay Medium (solid)	3 bottles
Sterile Water	3 bottles
VB12 Strain (take Y mL)	3 bottles
Loading slot	3 pieces
Empty plate frame	1 piece
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other materials required but not supplied

1. Sterile bench (sterile working for recommended)
2. Microtiter plate reader 610 - 630 nm (540 - 550 nm)
3. Incubator with dark chamber, 37 ℃ (98.6 ℉)
4. Water bath heatable to 95 ℃ (203 ℉)
5. pH meter; sterile syringe
6. Centrifuge > 8000 x g (if the sample cannot be filtrated)
7. Sterile tips for graduated micropipette 20 - 200 µL and 100 - 1000 µL, sterile graduated centrifuge vials with screw cap (15 and 50 mL) and sterile reaction vials 1.5 or 2.0 mL
8. 500 mL screw glass jar and volumetric flasks (100 and 1000 mL), 100 mL beaker
9. Sterile filters polyethersulfon 0.2 µm with syringe
10. Deionized water for sample extraction

Note

1. Warning and precautions for the user – the assay medium could evoke irritations of mucosa, eyes and skin – after running the test the strips used must be disposed of according to regulations (e.g. autoclaved)
2. Storage instructions Store the kit/reagents at 2-8 ℃ (35.6-46.4 ℉). Use the prepared reagents (standard, medium) immediately and reject them after the assay.
3. No quality guarantee can be assumed after expiration of the shelf life.
4. Each reagent is optimized for use in the E-FS-M002. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-M002 with different lot numbers.
5. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.

Storage and expiry date

Store the kit at 2-8℃. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8℃.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. Sample pretreatment Notice:

Experimental apparatus should be clean, and the disposable pipette should be disposable to avoid cross-contamination during the experiment.

2. Sample preparation

(1) Samples should be stored protected from light at 4 ℃ (39.2 ℉). Standard and samples should be run in triplicate. Sample extracts have to be used within one day and should be stored in the dark until analyzing.

(2) Sample extraction is carried out with 1 g (mL) homogenized sample in 40 mL sterile water or deionized water or extraction solution. **This equals a sample extraction dilution factor of 40**. This factor is already included in the standard curve (see Quality Assurance Certificate). For low VB12 concentrations a sample weight of up to 5 g (mL) can be used (this has to be considered in the evaluation).

(3) Only sterile sample extracts or sterile dilutions thereof should be pipetted onto the microtiter plate.

(4) Dilutions have to be prepared with sterile water from the test kit. Therefore after the sample extraction sterile working conditions and sterile consumables are necessary.

(5) A sterile filtration of the sample or the sample extract is always necessary for: samples like fruit juices and fitness drinks, which are not heated during sample extraction (except when the sample is heated 30 min at 95 ℃ (203 ℉) in a water bath.

(6) The extracted samples have to be diluted with sterile water from the test kit (the assay medium has to be always filtered).

2.1 Liquid samples with VB12 (multivitamin juices, fitness drinks)

(1) Add 1 mL sample into a 50 mL sterile centrifuge and fill up exactly to 40 mL with deionized water.

(2) Thereafter shake. Using a sterile filter (0.22 μ m) Filter the mixed solution to obtain sample solution A.

(3) Depending on the concentration range of sample solution A, further dilute sample solution A in 1.5 mL (or 2.0 mL) sterile reaction vials with **Sterile Water** from the test kit.

2.2 Solid samples with VB12 (milk powder, baby food and flour)

(1) Weigh 1 g (mL) homogenized sample into a 50 mL sterile vial, add about 40 mL of deionized water.

(2) Extract for 30 minutes at 95 ℃ (203 ℉) in a water bath.

(3) During extraction the vial has to be shaken well at least five times. It is important to make sure that the sterile vial is tightly closed.

(4) Chill down quickly to below 30 °C (86 °F). Using a sterile filter (0.22 μ m) Filter the mixed solution to obtain the filtrate, then centrifuge at 8000 rpm for 5min to obtain solution sample A.

(5) Depending on the concentration range of sample solution A, further dilute sample solution A in 1.5 mL (or 2.0 mL) sterile reaction vials with **Sterile Water** from the test kit.

3. Sample Dilution

(1) Example for the dilution factors of the sample extract
Sample with a konwn concentration of 1.2 μg/100 g

(2) The concentration of sample should be in the middle of the standard curve.
Therefore, the concentration is divided by standard 2 (0.06 μg/100 g).

(3) Calculation:
1.2 μg / 0.06 μg = 20→ dilution factor 20 (1:19)

(4) Dilution steps:
a) dilution factor 10→ 50 μL sample solution A + 450 μL sterile water from the test kit
c) dilution factor 2→ 250 μL from a + 250 μL of **Sterile Water** from the test kit.
Note: Dilution ratio ≤10 in each step.

4. Solution preparation

4.1 Standard preparation

(1) The bottle with sterile water: push the coloured lid up, pull off right up to the glass rim and turn entire lid to remove it. VB12 standards should be dissolved and diluted freshly.

(2) Open the VB12 standard bottle, place the lid down with the opening facing upwards.

(3) Add X mL **Sterile Water** (from the test kit) to the standard bottle (the X value can be found on the standard label). Close the standard bottle with the lid and dissolve the standard by shaking = *standard concentrate.*

(4) take 6 sterile vials (1.5 or 2.0 mL) and prepare from the dissolved standard concentrate a standard curve according to the following scheme:

standard curve* in μg / 100 g (mL)	sterile water μL		standard concentrate μL		total volume μL
blank: 0	500	+	0	=	500
standard 1: 0.03	475	+	25	=	500
standard 2: 0.06	450	+	50	=	500
standard 3: 0.09	425	+	75	=	500
standard 4: 0.12	400	+	100	=	500
standard 5: 0.18	350	+	150	=	500

Dilution factor: 40

4.2 Strain solution preparation

(1) Open the **VB12 Assay Medium** bottle and remove the desiccant using tweezers (discard the desiccant).

(2) Add 10 mL of **Sterile Water** from the test kit to the **VB12 Assay Medium** bottle.

(3) Close the VB12 assay medium bottle carefully and shake well.

(4) Heat the bottle in a water bath at 95 ℃ (203 ℉) for 5 min, shaking at least twice; always make sure that the bottle is tightly closed.

(5) Quickly chill down to room temperature below 30 ℃ (86 ℉) filter the *assay medium solution* through a 0.22 μm filter into a sterile 15 mL sterile vial.

(6) Take out the freeze-dried **VB12 Strain**, push the cap upward in the direction of the arrow, and pull the aluminum plastic cap counterclockwise.

(7) Remove the entire aluminum plastic cap, and it is recommended to use a 2 mL sterile syringe to add 1mL of **Sterile Water** from the test kit into the **VB12 Strain** bottle through the bottle stopper. Shake and mix well.

(8) Add Y mL (the Y value can be found on the standard label) of VB12 Strain solution form step 7 into the *assay medium solution*. Shake and mix well to form the test *strain solution* (the test Strain solution must be prepared and used immediately).

Note: To prevent laboratory contamination, the remaining bacteria in the strain bottle should be promptly cleaned out of the laboratory or autoclaved; If there is any residue in the prepared test bacterial solution, it is also necessary to clean it out of the laboratory or autoclave it in a timely manner.

Assay procedure

(1) Only sterile samples which are diluted with sterile water from the test kit should be pipetted onto the microtiter plate.

(2) Remove the required strips of the microtiter plate and place them into the additional holder; return the unused strips together with the desiccant to the foil bag and seal it well, store at 2 - 8 ℃ (35.6 - 46.4 ℉).

(3) Pipette 140 µL **standard** or **sample solution** into the assigned wells (flush the pipette tip with standard or sample solution) and pipette 140 µL of *strain solution* into the wells.

(4) Cover the plate with plate sealer. Incubate at 37 ℃ (98.6 ℉) in the dark for 44 - 48 h in an incubator

(5) Place the microtiter plate upside down on a table and dissolve the microorganisms thoroughly by shaking the plate on the surface of the desk

(6) Invert the plate to the regular position and remove the plate sealer.

(7) Destroy any bubbles on the surface of liquid in the wells (by means of a pipette tip or a needle)

(8) Measure the OD with a microtiter plate reader at 610 - 630 nm (alternatively at 540 - 550 nm)

Note: After 44 - 48 h of incubation, the microtiter plate can be stored for max. 48 h in the refrigerator, thereafter the OD should be measured.

Result analysis

(1) A 4-parameter evaluation is recommended, the test evaluation is correct on condition that

- OD blank < OD standard 1

(2) The sample dilution factor of 40 is already included in the standard curve. In the below formula merely the further dilution factor of the extract and a differing sample weight need to be taken into consideration.

(3) **For example:**

$$\text{VB12 (µg/ 100 g)} = \frac{\text{conc.standard curve} * \text{dilution factor}}{\text{sample weight ing}} =$$

Example:

Sample weight: 1.0 g

Dilution of the **sample:** 40 (no need to considered)

Dilution of the **sample solution A**: 20 (has to be considered)

Measured concentration from the Standard curve: VB12----0.12 µg/100 g

Actual concentration of the sample: 0.12 µg * 20/ 1 g = 2.4 µg/100 g