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Manual

ID-Vit® vitamin B_{12}

Microbiological test kit for the determination of vitamin B_{12} in serum using a Lactobacillus delbrueckii subsp. lactis coated microtitre plate

For use in human and veterinary medicine and in research

Valid from 2019-05-22

REF **KIF012**

Σ 96

+8°C +2°C

IVD

CE

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1. INTENDED USE

ID-Vit® Vitamin B_{12} is a microtiter plate test kit based on a microbiological method which measures the total vitamin B_{12} content in serum. The test kit contains the standard and all reagents required to perform the test. It is sufficient for 96 determinations including standard curves. An ELISA reader is required for the evaluation of the results. For use in human and veterinary medicine and in research. For *in vitro* diagnostic use only.

2. INTRODUCTION

Vitamin B_{12} (cobalamin), a collective term for a group of various substituted corrinoide with cobalt as the central atom, is found free and also protein-bound in food. The protein-bound form is degraded by pancreatic protease, releasing free B_{12} which binds to intrinsic factor, a protein secreted by gastric parietal cells of the stomach mucosa. The cobalamin-intrinsic factor complex is bound to mucous membrane cells of the stomach in the ileum and absorbed by the cells. In the case of high doses, a diffusion of the complex also takes place. Vitamin B_{12} is bound to the protein transcolbalamin II (TC-II) within the cells. TC-II serves as a transport protein for vitamin B_{12} in the circulation system.

Vitamin B_{12} is involved as a co-enzyme in metabolic processes and plays an important role in the formation of the blood, the development of the nervous system and the regeneration of the mucous membranes. In addition, there is a direct relationship to the formation of folic acid because methylcobalamin is involved in the transfer of methyl groups for the synthesis of methionin from homocystein.

Vitamin B_{12} deficiency

Vitamin B_{12} deficiency is rarely caused by dietary factors. In most cases, it results from a resorption disorder of the intestines or defective development of intrinsic factor. Since vitamin B_{12} resorption can be reduced up to 50 % in the elderly, an increased supplement is recommended. Pregnant women with a lacto-vegetarian diet are also recommended to increase their intake because their vitamin B_{12} stores in the liver could be exhausted.

The classical vitamin B_{12} deficiency disease is pernicious anemia. In the early stages of the disease, vitamin B_{12} deficiency symptoms are manifested as weariness, palpitations, pallor or jaundice.

Indications for vitamin B_{12} determination

- Megaloblastic (pernicious) anemia
- Hyperhomocysteinaemia (patients on dialysis, old people)
- Homocysteinurie

- Peripheral neuropathy
- Patients with CED, gastritis, gastrectomy, gluten intolerance or intestinal resorption disorders
- Pancreatic insufficiency
- Patients with thrombosis
- Alcoholism
- Chronic liver and kidney disease
- Vitamin B_{12} deficiency from diet (vegan vegetarians)
- Pregnancy and lactation

3. PRINCIPLE OF THE TEST

The serum samples are pre-treated and diluted with a buffer mixture, and then transferred into the wells of a microtiter plate coated with *Lactobacillus delbrueckii* subsp. *lactis*. The addition of vitamin B_{12} in either standards or samples gives a vitamin B_{12}-dependent growth response until vitamin B_{12} is consumed. After incubation at **37 °C** for **48 h**, the growth of *Lactobacillus delbrueckii* subsp. *lactis* is measured turbidimetrically at 610–630 nm (alternatively at 540–550 nm) in an ELISA reader and a standard curve is generated from the dilution series. The amount of vitamin B_{12} is directly proportional to the turbidity.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KIF012	PLATE	*Lactobacillus delbrueckii* subsp. *lactis*-precoated microtiter plate	1 plate
KIF012	SOL	Sample preparation buffer	4 x 5 ml
KIF012	STAB	Stabilizer, lyoph.	4 x
KIF012	DIL	Water	4 x 30 ml
KIF012	ASYMED	Vitamin B_{12} assay medium	4 x
KIF012	STD	Vitamin B_{12} standard, lyoph.	4 x
KIF012	FOL	Adhesive cover foil	4 x
KIF012	FRA	Replacement holder for 96 well plates	1 x
KIF012	CTRL1	Vitamin B_{12} control 1, lyoph.	4 x
KIF012	CTRL2	Vitamin B_{12} control 2, lyoph.	4 x

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Incubator with a dark incubation chamber, 37 °C
- Water bath (90 °C–100 °C)
- optional for sample preparation: thermoblock (95 °C)
- ELISA reader 610–630 nm (540–550 nm)
- Calibrated precision pipettors and 20–1000 µl tips
- Pipettes of 5 and 10 ml
- 1,5–2 ml reaction vials, sterile
- 0,2 µm sterile polyethersulfone (PES) filter with a sterile disposable syringe
- 15 ml centrifuge tubes, sterile (e. g. Falcon tubes)
- Biocentrifuge (10 000 *g*)

6. PRECAUTIONS

- As the test is based on a microbiological method, the general guidelines for sterile work should be observed as far as possible, (preferably work in a sterile bench / PCR hood, use of sterile instruments or equipment).
- GLP (Good Laboratory Practice) guidelines have to be observed.
- Water quality is extremely important for the test. Only the water delivered with the test kit [DIL] should be used.
- For sterile filtration, only a sterile polyethersulfone filter must be used.
- It is essential to run a standard curve for each separate assay.
- Controls should be measured with each assay.
- We recommend measurements in duplicate.
- If a higher dilution results in a higher measured value, inhibitors like antibiotics might be present.
- Reagents should not be used beyond the expiration date shown on the label.
- Wear gloves during the test.
- Used microtiter stripes [PLATE] and materials that have been in contact with patient‘s samples should be handled and disposed as potentially infectious.

7. STORAGE AND PREPARATION OF REAGENTS

- Store test kit and reagents at 2–8 °C.
- Prepare reagents freshly and use them immediately after preparation. Discard remaining unused reagents and waste in accordance with country, federal, state, and local regulations.
- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. Prepare only the appropriate amount necessary for each run. The kit can be used up to 4 times within the expiry date stated on the label.

7.1 Water

- Water [DIL] (for medium [ASYMED], standard [STD] and controls [CTRL1, CTRL2])
- Push the cap up and pull it back to the rim of the glass, then twist the whole cap off.

7.2 Preparation of the stabilisation reagent

- Add 4 ml sample preparation buffer [SOL] to a vial of lyophilised stabilisation reagent [STAB], then homogenise using a vortex.
- Stabilisation reagent cannot be stored.

7.3 Preparation of the controls

- The lyophilised controls [CTRL1, CTRL2] have to be resuspended with each 300 µl water [DIL] from the test kit, then homogenise using a vortex.
- After reconstitution, the controls are treated like samples.
- The concentration of the controls changes from lot to lot and is stated in the product specification.

7.4 Preparation of the standard curve

- For the preparation of the standard curve, standard concentrate is needed. To prepare standard concentrate, resuspend the lyophilised standard [STD] with x ml (x = please see the enclosed quality control protocol for the volume needed) water [DIL] supplied with the test kit, then homogenise using a vortex.

- In 6 sterile reaction tubes (1.5–2 ml volume), prepare a standard curve from standard concentrate and water [DIL] following the scheme depicted in the table below:

Vitamin B_{12} [ng/l]	Water [DIL] [µl]	+	Standard concentrate [µl]	=	Total volume [µl]
Blank: 0	700	+	0	=	700
Standard 1: 6	700	+	50	=	750
Standard 2: 18	400	+	100	=	500
Standard 3: 27	350	+	150	=	500
Standard 4: 36	300	+	200	=	500
Standard 5: 54	200	+	300	=	500

7.5 Preparation of the sterile assay medium

- Fresh sterile assay medium has to be prepared each time before performing a test.
- Remove lyophilised assay medium from the desiccant bag in the assay medium bottle by taking the bag with a forceps and shaking it whilst still inside the bottle. Then remove the clean desiccant bag and discard it.
- Add 10 ml water [DIL] to the assay medium bottle [ASYMED], close the bottle firmly and shake it. This amount is sufficient for 6 microtiter stripes.
- Heat the medium bottle in a water bath at 90–100 °C for 5 min, shake well at least 2 times during this incubation time. Take care that the medium bottle is always firmly closed.
- Quickly cool the medium bottle to < 30 °C (at 2–8 °C for 10 min).
- Filter the medium using a disposable syringe (10 ml) and the 0,2 µm PES filter into a sterile centrifuge tube (15 ml, e. g. Falcon).
- After this preparation, the assay medium can be used in the test.

7.6 Microtiter plate [PLATE]

- Store the microtiter plate [PLATE] in the aluminium packaging containing the desiccant bag at 2–8 °C.
- The microtiter plate [PLATE] has to be protected from humidity and contamination.

- Take care that the aluminium packaging is not damaged.
- Carefully close the aluminium packaging after opening.
- Take only the microtiter stripes needed directly before usage to avoid contamination

8. SAMPLE STORAGE AND PREPARATION

- Use serum for analysis.
- Samples are stable at 2–8 °C for one day in the dark. For longer storage, samples should be frozen and kept at -20 °C.
- Hemolytic samples may give erroneous results and should not be used for analysis. Lipemic samples should be centrifuged at 13 000 *g* before assaying to obtain fat free serum as far as possible.
- Samples should be centrifuged (at least 5 min at 10 000 *g*) prior to measurement. Use the resulting supernatant in the test.

8.1 Sample pretreatment

Add 75 µl serum/control to 300 µl of prepared stabilising solution (ratio 1:5), mix, heat to 95 °C for 30 min and then cool quickly (10 min at 2–8 °C). Afterwards, centrifuge for 10 min at 10 000 *g*.

8.2 Sample dilution

Take 100 µl from the supernatant of the prepared serum/control, add 400 µl water [DIL] and mix. The sample treatment and dilution result in a total dilution of 1:25 (= sample dilution factor).

9. ASSAY PROCEDURE

9.1 Test preparations

Take as many microtiter strips as needed from kit. Return unused strips and any unused test kit component to the original packaging, and put in the refrigerator. Bring all necessary reagents to room temperature.

9.2 Test procedure

- Take as many microtiter strips as needed from the kit and put them in the second microtiter strip holder [FRA].
- Put 150 µl sterile assay medium into the cavities.
- Add each 150 µl of the prepared standard curve, samples and controls into the respective cavities. Pre-rinse each pipet tip with standard, control or sample solution, respectively.
- Carefully seal the plate with adhesive cover foil [FOL]. Important: the cavities must be made airtight by pressing the foil down with the hand!
- Keep at 37 °C for 48 h in an incubator.

9.3 Measurement

- Press the adhesive cover foil [FOL] firmly down again with the hand.
- Upturn the microtiter plate [PLATE], put it onto a tabletop and shake the microbes well.
- Turn the microtiter plate [PLATE] over again and carefully remove the adhesive cover foil [FOL]. During this, fix the strips in the frame with your hand because the foil is highly adhesive.
- Remove air bubbles in the cavities using a pipet tip or a needle.
- Read turbidity in an ELISA reader at E 610–630 nm (alternatively at 540–550 nm).

Please note

- After 48 h incubation time, the microtiter plate [PLATE] may be stored for a maximum of 48 h in the refrigerator before measuring the turbidity.
- To prevent time-loss through public holidays or weekends, the microtiter plate [PLATE] may also be measured after 60 h incubation.

10. EVALUATION OF RESULTS

We recommend to use the 4 parameter algorithm to calculate the results. The sample dilution factor has to be considered for data evaluation.

The blank should have an optical density < standard 1. It serves as optical control to exclude contaminations and is not included in the calculation of results.

10.1 Calculation

Vitamin B_{12} in ng/l = value from the standard curve × sample dilution factor (25)

Reference value for human serum

Based on studies of matrix samples of apparently healthy persons (n = 83) the following values were estimated.

Vitamin B_{12}:

25–500 ng/l (≙ 18.4–368.5 pmol/l)

Please note

A concentration range of 150–1350 ng/l vitamin B_{12} is covered at a sample dilution of 1:25.

We recommend each laboratory to develop its own normal range as normal ranges strongly depend on the choice of the patient collective. The values mentioned above are only for orientation and can deviate from other published data.

10.2 Quality control

The extinction of the highest standard has to be > 0.6.

Results, generated from the analysis of control samples, should be evaluated for acceptability. The results for the samples may not be valid if within the same assay one or more values of the quality control sample or the highest standard are outside the acceptable limits.

11. LIMITATIONS

Whole blood, EDTA plasma, and heparin plasma cannot be used in the assay.

12. PERFORMANCE CHARACTERISTICS

The following performance characteristics have been collected using human serum samples.

12.1 Precision and reproducibility

Intraassay (n = 21)

	Vitamin B_{12} [ng/l]	**CV [%]**
Sample	294	5.38

Interassay (n = 3)

	Vitamin B_{12} [ng/l]	CV [%]
Sample	285	8.0

12.2 Recovery

Samples from 3 patients were spiked with vitamin B_{12} and analysed. The mean values are shown below.

Sample (n=5)	**Mean value original sample [ng/l]**	**Spike [ng/l]**	**Vitamin B_{12} expected [ng/l]**	**Vitamin B_{12} measured [ng/l]**	**Recovery Rate [%]**
A	566.58	187.5	754.08	726.36	85
		375.0	941.58	908.21	91
				Recovery rate in total [%]	**88**

Sample (n=5)	**Mean value measured in original sample [ng/l]**	**Spike [ng/l]**	**Vitamin B_{12} expected [ng/l]**	**Vitamin B_{12} measured [ng/l]**	**Recovery Rate [%]**
B	481.3	187.5	668.8	681.45	107
		375.0	856.3	929.50	120
				Recovery rate in total [%]	**114**

Sample (n=5)	Mean value measured in original sample [ng/l]	Spike [ng/l]	Vitamin B_{12} expected [ng/l]	Vitamin B_{12} measured [ng/l]	Recovery Rate [%]
C	526.44	187.5	713.94	762.23	126
		375.0	901.44	845.02	85
			Recovery rate in total [%]		**105**

13. REFERENCES

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14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- *ID-Vit*® is a trademark of Immundiagnostik AG.

- Reagents should not be used beyond the expiration date stated on the kit label.
- Do not interchange different lot numbers of any kit component within the same assay.
- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.
- Control samples should be analysed with each run.
- The assay should always be performed according to the enclosed manual.

Used symbols: