Manual

ID-Vit® vitamin B_{12}

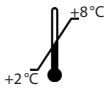
Microbiological test kit for the determination of vitamin B_{12} in serum using a Lactobacillus delbrueckii subsp. lactis coated microtiter plate

For use in human and veterinary medicine and in research

Valid from 2025-07-24

REF **KIF012** Σ 96

REF **KIF012.2** Σ 2 x 96



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Safety information

The assay has to be performed exclusively according to the instructions for use enclosed with the kit. Important safety information for this product can be found in the chapter WARNINGS AND PRECAUTIONS.

Table of Contents

1. INTENDED PURPOSE

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. 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. In the distal Ileum, the cobalamin-intrinsic factor complex is bound to special receptors and thus absorbed into the intestinal mucosa cells. In the case of high doses, a diffusion of free vitamin B_{12} also takes place. Within the cells, vitamin B_{12} is released from the intrinsic factor and bound to the protein transcolbalamin II (TC-II). 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 to folic acid, the body also needs vitamin B_{12} as an essential cofactor to break down homocysteine.

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 (vegans)
- Pregnancy and lactation

3. PRINCIPLE OF THE TEST

The serum samples are pre-treated with a buffer mixture and diluted, 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 **46–50 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	KIF012.2
KIF000.30	DIL	Water	4 x 30 ml	5 x 30 ml
KIF012/ KIF012.2	PLATE	microtiter plate, precoated with *Lactobacillus delbrueckii* subsp. *lactis*-	1 x	2 x
	SOL	Sample preparation buffer	6 x 5 ml	12 x 5 ml
	ASYMED	Vitamin B_{12} assay medium	4 x	4 x
	STD	Vitamin B_{12} standard; lyophilised	4 x	3 x
	FOL	Adhesive cover foil	1 x whole 3 x half	3 x whole
	FRA	Replacement holder for microtiter strips	1 x	1 x
	CTRL1	Vitamin B_{12} control 1; lyophilised	4 x	3 x
	CTRL2	Vitamin B_{12} control 2; lyophilised	4 x	3 x

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Incubator with a dark incubation chamber, 37 °C
- Water bath (90 °C–100 °C) or thermoblock (95 °C)
- ELISA reader 610–630 nm (540–550 nm)
- Calibrated precision pipettors and sterile single use 20–1 000 µl tips
- 5 ml and 10 ml pipets
- 1.5–2 ml reaction vials
- 0.2 µm sterile polyethersulfone (PES) filter with a disposable syringe (10 ml)
- 15 ml centrifuge tubes (e. g. Falcon tubes)
- Biocentrifuge (10 000 *g*)
- Vortex

6. WARNINGS AND PRECAUTIONS

- The test is based on a microbiological method. Contaminations lead to erroneous results.
- Water quality is extremely important for the test. Use only the water delivered with the test kit (**DIL**).
- For sterile filtration, only a sterile polyethersulfone filter must be used.
- It is essential to run a standard curve for each separate assay.
- Measure controls with each assay.
- We recommend measurements in duplicate.
- Do not use reagents beyond the expiration date shown on the label.
- As a precaution, it is recommended that the human material used is always considered potentially infectious.
- Used microtiter stripes and materials that have been in contact with patient samples must be handled and disposed of 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.
- 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 3 x (KIF012.2) or 4 x (KIF012) within the expiry date stated on the label.

7.1 Water

- Water (**DIL**) for medium (**ASYMED**), standard (**STD**) , controls (**CTRL1**, **CTRL2**) and dilutions.

7.2 Preparation of the sterile assay medium

- Fresh sterile assay medium has to be prepared each time before performing a test.
- Remove the desiccant bag from the lyophilised 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 vortex well. This amount is sufficient for 6 microtiter stripes.
- Filter the medium using a disposable syringe (10 ml) and the 0.2 µm PES filter into a centrifuge tube (15 ml, e. g. Falcon).
- After this preparation, the sterile assay medium can be used in the test.

Note: Any suspended solids present in the assay medium, which are removed by the sterile filtration, have no impact on the measured values.

7.3 Preparation of the controls

- The lyophilised controls (**CTRL1**, **CTRL2**) have to be resuspended each with **x µl** water (**DIL**) (x = see product specification) from the test kit, then homogenise using a vortex.
- 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** water (**DIL**) (x = see quality control protocol) supplied with the test kit, then homogenise using a vortex.
- Prepare a standard curve in 6 sterile reaction tubes (1.5–2 ml volume) 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 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 can be frozen and kept at -20 °C for up to 5 months.
- Centrifuge samples prior to measurement (at least 5 min at 10 000 *g*). Use the resulting supernatant in the test.
- Do not use hemolytic samples for analysis as they may give erroneous results. Centrifuge lipemic samples at 13 000 *g* for 10 min before assaying to obtain a serum that is as fat free as possible.

8.1 Control dilution

Add 480 µl water (**DIL**) to 20 µl control and mix. This corresponds to a 1:25-dilution (= sample dilution factor).

8.2 Sample pretreatment

Mix 75 µl serum with 300 µl sample preparation buffer (**SOL**) (ratio1:5), vortex, heat at 95 °C for 30 min, then cool 10 min at 2–8 °C and then centrifuge for 10 min (10 000 *g*).

8.3 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 the kit. Return unused strips and any unused test kit components to the original packaging, and store 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 each cavity.

- Add 150 µl of the prepared standard dilutions (blank, standard 1–5), 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 **46–50 h** in an incubator.

9.3 Measurement

- Press the adhesive cover foil (**FOL**) firmly down again with the hand.
- Turn the microtiter plate (**PLATE**) upside down, place 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 E 540–550 nm).

Please note

- After 46–50 h incubation time, the microtiter plate (**PLATE**) may be stored for a maximum of 48 h in the refrigerator before measuring the turbidity.

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 serves as a visual control to exclude contamination and is not taken into account in the calculation. The optical density must be < standard 1. If this is not the case, the analysis must be carried out again.

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 = 62) the following values were estimated.

Vitamin B_{12}:

200–830 ng/l (≙ 147.2–626.5 pmol/l)

Please note

A concentration range of 150–1 350 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 reference range is given for guidance only and may differ 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

Only serum can be used for the test.

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. GENERAL NOTES ON THE TEST

- 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.
- Do not use reagents beyond the expiration date stated on the kit label.
- Do not interchange different lot numbers of any kit component within the same assay.
- Follow the guidelines for medical laboratories.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which has not been consulted 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 made within 14 days after reception of the product. The product should be sent to Immundiagnostik AG along with a written complaint.
- Analyse controls with each run.
- Always perform assay according to the enclosed manual.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.

14. DISPOSAL

Liquid test components, microtiter plates and vials should be treated as ordinary laboratory waste unless otherwise stated. Specimens and other potentially infectious materials must be disposed of in accordance with regulatory requirements.

15. REFERENCES

1. Strohecker R, Henning H (1963) Vitamin-Bestimmungen. Hrsg. E. Merck AG, Darmstadt. *Verlag Chemie*, Weinheim
2. Kräutler, B., 2005. Vitamin B12: chemistry and biochemistry. *Biochemical Society transactions*, **33**, pp.806–810.
3. Obeid, R. & Herrmann, W., 2006. Mechanisms of homocysteine neurotoxicity in neurodegenerative diseases with special reference to dementia. *FEBS letters*, **580**(13), pp.2994–3005.
4. Partearroyo, T. et al., 2013. Vitamin B12 and Folic Acid Imbalance Modifies NK Cytotoxicity, Lymphocytes B and Lymphoprolipheration in Aged Rats. *Nutrients*, **5**(12), pp.4836–4848.
5. Schwarz, J. et al., 2014. The influence of a whole food vegan diet with Nori algae and wild mushrooms on selected blood parameters. *Clinical laboratory*, **60**(12), pp.2039–2050.
6. Schwarz, J. et al., 2015. Biochemical Identification of Vitamin B12 Deficiency in a Medical Office. *Clinical laboratory*, **61**(7), pp.687–692.

16. SYMBOLS

Used symbols:


Temperature limitation

REF Catalogue number

IVD In Vitro Diagnostic Medical Device

→REF To be used with


Manufacturer

Contains sufficient for <n> tests

LOT Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet


Do not re-use

UDI Unique Device Identification

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