

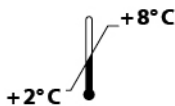
ID-Vit® Vitamin B₁₂ Assay Kit

Microbiological test kit for the determination of vitamin B₁₂ in serum using a Lactobacillus delbrueckii subsp. lactis coated microtitre plate

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REF

KIF012



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1. INTENDED USE

ID-Vit® Vitamin B₁₂ is a microtiter plate test kit based on a microbiological assay which measures the total Vitamin B₁₂ content in serum. The test kit contains all required reagents, e.g. standard, medium and microtiter plate coated with a specific microorganism, sufficient for 96 determinations including standard curves. An ELISA reader is required for evaluation of the Vitamin B₁₂ content. For use in human and veterinary medicine and in research. The Vitamin B₁₂ Assay Kit is for research use only.

2. INTRODUCTION

Vitamin B₁₂ (Cobalamin), a collective term for a group of various substituted corrinoids 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 B₁₂, 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 B₁₂ is bound to the protein Transcobalamin II (TC-II) within the cells. TC-II serves as a transport protein for Vitamin B₁₂ in the circulation system.

Vitamin B₁₂ is involved in the metabolism process as a co-enzyme, 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 B₁₂ -Deficiency

Vitamin B₁₂ deficiency is seldom caused by dietary factors. In most cases, it results from a re-sorption disorder of the intestines or defective development of intrinsic factor. Since vitamin B₁₂ resorption could 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 the intake because their liver vitamin B₁₂ stores may be exhausted.

The classical vitamin B₁₂ deficiency disease is pernicious anemia. In the early stages of the disease, vitamin B₁₂ deficiency symptoms are manifested as weariness, palpitations, pallor or jaundice.

Indications for Vitamin-B₁₂-determination

- Megaloblastic (pernicious) anemia
- Hyperhomocysteinaemia (patients on dialysis, old people)
- Homocysteinuria
- 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 B₁₂ deficiency from diet (vegan vegetarians)
- Pregnancy and lactation

3. PRINCIPLE OF THE TEST

The serum is pre-treated and diluted with a buffer mixture, and then samples were transferred in the wells of a microtiter plate [PLATE] coated with *Lactobacillus delbrueckii subsp. lactis*. The addition of vitamin B₁₂ in either standards [STD] or samples gives a vitamin B₁₂-dependent growth response until vitamin B₁₂ is consumed. After incubation at **37°C for 44 - 48 h**, the growth of *Lactobacillus plantarum* 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 B₁₂ is directly proportional to the turbidity.

4. MATERIAL SUPPLIED

Catalog No	Label	Kit Components	Quantity
KIF012MTP	PLATE	One <i>Lactobacillus delbrueckii subsp. lactis</i> -precoated microtiter plate, ready to use	12 x 8 wells
KIF012SO	SOL	Sample preparation buffer 5 ml, ready to use	4 x
KIF012STAB	STAB	Stabilizer	4 x
KIF012DI	DIL	Water 30 ml	4 x
KIF012ME	ASYMED	Vitamin B ₁₂ assay medium	4 x
KIF012ST	STD	Vitamin B ₁₂ standard	4 x
KIF012FO	FOL	Cover plastic foil	4 x
KIF012FR	FRA	Replacement holder for 96-well plates	1 x
KIF012KO1	CTRL1	Control 1 Vitamin B ₁₂	4 x
KIF012KO2	CTRL2	Control 2 Vitamin B ₁₂	4 x

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Incubator with a dark incubation chamber, 37 °C
- Water bath (90°C - 100°C)
- ELISA-Reader 610 - 630 nm (540 - 550 nm)
- Micropipette 20 - 200 µl
- Micropipette 100 -1000 µl
- Micropipette tips to deliver 20 - 200 µl and 100 -1000 µl, sterile
- Pipettes of 5 and 10 ml
- 1,5 - 2 ml reaction vials, sterile
- 0,2 µm sterile polyethersulfone filter with a sterile tip
- 15 ml centrifugal tubes, sterile (e.g. Falcon tubes)
- Biocentrifuge (10 000 x g)

6. PREPARATION AND STORAGE OF REAGENTS

- Store test kit / reagents at 2-8°C.
- Prepare reagents freshly and use immediately after preparation. Discard remaining unused reagents and waste in accordance with country, federal, state, and local regulations.
- Put unused reagents (standard, controls, medium) in the test kit and store at 2-8°C.
- Store unused strips in the original package with dry bag securely closed at 2-8°C to prevent contamination or moisture exposure.
- No warranty can be given after the expiry date (see label of test package).
- To run assay more than once, ensure that reagents are stored at conditions stated on the label. Prepare only the appropriate amount necessary for each assay. The kit can be used up to 4 times within the expiry date stated on the label.

7. PRECAUTIONS

- As the test is based on a microbiological method, the general guidelines for **sterile work** must be observed as far as possible, (work in a sterile bench, PCR-Hood, use of sterile instruments or equipment).
- GLP (Good Laboratory Practice)-guidelines should be observed.
- **Water quality** is extremely important. Only the water delivered with the test kit [DIL] should be used for medium dilution [ASYMED], standard [STD] and control [CTRL1, CTRL2] reconstitution as well as for sample preparation.
- For sterile filtration, only a sterile polyethersulfone filter must be used.
- It is essential to run a standard curve for each separate assay.
- It is recommended to run a duplicate standard [STD] curve as well as a sample and control [CTRL1, CTRL2] analysis.
- 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 kit label.
- Wear gloves during the test.
- Used microtiter plates [PLATE] and materials that have been in contact with patient's samples should be handled and disposed as potentially infectious.
- Signs for reagent damage: The highest standard should have an absorption higher than 0.6 Extinction units (A_{630nm} > 0,6)

8. SAMPLE PREPARATION

Notes

- Patient serum is used for analysis.
- Original samples should be kept light-protected at 2-8°C until measurement. The samples are stable for 8 hours at 2-8°C 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 x g before assaying to obtain fat free serum as far as possible.
- Samples should be centrifuged (5 min at 10000 g) prior to measurement and the resulting supernatant used in the test.

8.1 Sample pretreatment

Add 4 ml sample preparation buffer [SOL] to the bottle with the stabilizer [STAB]. Mix well. Add 75 µl serum sample or control [CTRL1, CTRL2] to 300 µl of this solution, mix, heat to 95°C for 30 min and then cool quickly. Afterwards, centrifuge for 10 min at 10000 g.

8.2 Sample dilution

Take 100 µl from the supernatant of the treated serum sample and control [CTRL1, CTRL2], add 400 µl water [DIL] and mix. The sample treatment and dilution results in a final dilution of 1:25 (= sample dilution factor).

9. ASSAY PROCEDURE

Procedural notes

- Quality control guidelines should be observed.
- 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.
- The assay should always be performed according the enclosed manual.

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 foil bag, reseal them together with the desiccant provided, and put in the refrigerator. Bring all necessary reagents to room temperature.

Water [DIL] for medium [ASYMED], standard [STD] and controls [CTRL1, CTRL2]

Push the lid up, pull it back to the rim of the glass and then remove the entire seal by turning.

Assay medium [ASYMED]

- The medium must be freshly prepared before each test.
- Take the dry bag out of medium vial [ASYMED] by tweezers, shake off and discard.
- Add 10 ml of water [DIL] to the assay medium [ASYMED], securely close the bottle and shake well. The amount is sufficient for 6 strips.
- Heat the bottle with medium [ASYMED] in a water-bath at 90 - 100 °C for 5 min, while shaking well at least twice. It is important to make sure that the medium bottle [ASYMED] is firmly closed at all times.
- Quickly cool the medium bottle [ASYMED] to under 30 °C.
- Filter 10 ml medium [ASYMED] sterilely with a 0.2 µm filter in a centrifuge test tube (e.g. 15 ml, Falcon).

Controls [CTRL1, CTRL2]

- The controls must be freshly prepared before the test.
- Open the bottles of controls [CTRL1, CTRL2], remove seal. Dispose of screw-top lid and seal.
- Add 0.3 ml water [DIL] from the test kit to the control bottles [CTRL1, CTRL2], close the bottle and dissolve by vortexing the bottle (= control).
- Treat the controls afterwards as the sample is treated.
- Pipette 150 µl of the reconstituted controls [CTRL1, CTRL2] into each well. We recommend to run a duplicate.
- For the concentration of the controls [CTRL1, CTRL2] please see control specification.

Standard [STD]

Before the test, freshly prepare the standard curve solutions:

- Open the bottle of standard [STD], place the screw-top lid upside-down on the work bench.
- Add x ml (x = see QStest kit data sheet) water [DIL] from the test kit to the standard bottle [STD], close the bottle and shake (= standard concentrate).
- Add water [DIL] into 6 sterile reaction vials (capacity 1.5 – 2.0 ml) and then pipet the standard concentrate to the vials. Prepare a standard curve using the following

scheme:

Vitamin B ₁₂ [ng / l]	Water [DIL] [µl]	+	Standard [STD] [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

9.2 Test Initiation

- Take as many microtiter strips as needed from the kit in put them in the second microtiter strip holder [FRA]. Return unused strips to the original foil bag, reseal them together with the desiccant provided, and store at 2-8° C to prevent contamination or moisture exposure.
- A medium solution is sufficient for 6 strips.
- Put 150 µl Vitamin B₁₂ assay medium [ASYMED] in the cavities.
- Add 150 µl standard [STD], controls [CTRL1, CTRL2], respectively, sample in the cavities. Pre-rinse the pipette tip with standard, control and sample solution respectively.
- Carefully seal the plate with plastic foil [FOL]. Important: the cavities must be made airtight by pressing down with the hand!
- Keep at **37 °C** for 48 hrs in an incubator.

9.3 Measurement

- Securely press the foil [FOL] down with the hand.
- Upturn the plate [PLATE] onto a tabletop and shake the germination well.
- Turn the plate [PLATE] over again and carefully remove the foil [FOL], beginning with the upper right corner and pulling diagonally backwards at an angle of 180°. 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 pipette tip or a needle.
- Read turbidity in an ELISA-Reader at E610 - 630 nm (alternatively at 540 - 550 nm).

Please note

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

10. EVALUATION OF RESULTS

We recommend to use the 4-Parameter-algorithm to calculate the results. The sample dilution factor should be considered for data evaluation.

Serum

Vitamin B₁₂ in ng / l = Value from the standard curve x sample dilution factor

Reference value for human serum

Vitamin B₁₂ (n = 83): 25 - 500 ng/L (= 18.4 - 368.5 pmol/L)

Please note: A concentration range of 150 - 1350 ng/L Vitamin B₁₂ is covered at a sample dilution 1 : 25.

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

11. PERFORMANCE CHARACTERISTICS*Precision and reproducibility*

Intra Assay (n = 21)		
	Vitamin B ₁₂ [ng/L]	CV [%]
Sample	294	5.38
Inter-Assay (n = 3)		
	Vitamin B ₁₂ [ng/L]	CV [%]
Sample	285	8.0

Recovery

Samples from 3 patients were spiked with Vitamin B₁₂ and analyzed. The mean values are shown below:

n = 5

Sample	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 [%]
A	566.58	187.5	754.08	726.36	85
		375.0	941.58	908.21	91
Recovery rate in total [%]					88

n = 5

Sample	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

n = 5

Sample	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

12. REFERENCES

Strohecker R, Henning H (1963) Vitamin-Bestimmungen. Hrsg. E. Merck AG, Darmstadt. Verlag Chemie, Weinheim

Obeid R, Herrmann W (2006) Mechanism of homocysteine neurotoxicity in neurodegenerative diseases with special reference to dementia. *FEBS Lett* 29,580(13):2994-3005, Epub 2006 May 6

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Assay components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the test package are for research use only.
- Reagents should not be used after the date of expiry stated on the label.
- Single components with different lot numbers should not be mixed or exchanged.
- Guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure that are not coordinated with the producer may influence the results of the test.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident. Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.



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