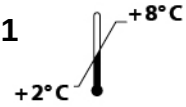
Manual

ID-Vit® *Vitamin B_1 Assay Kit*

Microbiological test kit for the determination of vitamin B_1 in whole blood using a Lactobacillus fermentum coated microtiter plate

Valid from 08.03.2012

REF **KIFR001**



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1. INTENDED USE

ID-Vit®*Vitamin B_1* is a microtiter plate test kit based on a microbiological assay which measures the Vitamin B_1 content in whole blood. 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 B_1 content. For use in human and veterinary medicine and in research. The Vitamin B_1 Assay Kit is for research use only.

2. INTRODUCTION

The bioactive form of vitamin B_1 is thiamin pyrophosphate. It plays an important role as a co-enzyme in carbohydrate and amino acid metabolism. Thiamine pyrophosphate is a vital co-factor for enzymes involved in several key metabolic processes in the nervous system, the heart, the blood cells, and the muscle. Vitamin B_1 assists in the conversion of carbohydrates into energy, necessary for healthy brain and nerve cells and heart function.

Vitamin B_1 deficiency

Vitamin B_1 deficiency may result from a deficiency in the diet. Eventually, a severe vitamin B_1 deficiency may lead to BERI-BERI, characterized by nerve, heart, and brain abnormalities. Deficiency may occur in alcoholics or in special clinical situations such as hemodialysis, chronic peritoneal dialysis, or after administration of glucose to a vitamin B_1-depleted patient. Further vitamin B_1 deficiency diseases are Wernicke's encephalopathy, Korsakow-syndrome, and some forms of Landry's paralysis. Also myopathie was found in relation to thiamine deficiency.

Indications for vitamin B_1 determination

- Suspicion of Vitamin B_1 deficiency
- Determination of the metabolic active Vitamin B_1
- Vitamin-B_1-supplementation of patients receiving total parenteral nutrition
- Disorders of the amino acid metabolism
- Malabsorption due to alcoholism
- Patients with suspicion of neuritis

3. PRINCIPLE OF THE TEST

The blood samples are enzymatically pre-treated and then transferred in the wells of a microtiter plate [PLATE] coated with ***Lactobacillus fermentum***. The addition of vitamin B_1 in either standards [STD], control [CTRL1, CTRL2] or samples gives a vitamin B_1-dependent growth response until vitamin B_1 is consumed. After incubation at **37 °C** for **48 h**, the growth of ***Lactobacillus fermentum*** 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_1 is directly proportional to the turbidity.

4. MATERIAL SUPPLIED

Catalog No	Label	Kit Components	Quantity
KIF001MTP	PLATE	One ***Lactobacillus fermentum***-precoated microtiter plate, ready to use	12 x 8 wells
KIF001SO	SOL	Sample preparation buffer 5 ml, ready to use	5 x
KIF001ENZ	ENZ	Enzyme, lyophilized	5 x
KIF001DI	DIL	Water 30 ml	4 x
KIF001ME	ASYMED	Vitamin B_1 assay medium	4 x
KIF001ST	STD	Vitamin B_1 standard	4 x
KIF001KO1	CTRL1	Vitamin B_1 control 1	4 x
KIF001KO2	CTRL2	Vitamin B_1 control 2	4 x
KIF001FO	FOL	Cover plastic foil	4 x
KIF001FR	FRA	Replacement holder for 96-well plates	1 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, medium, controls) 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 and controls [CTRL1, CTRL2] as well as samples.
- 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 Extinktion units (A_{630nm} > 0,6).

8. SAMPLE PREPARATION

- Venous fasting blood samples are suited for this test system. EDTA-whole-blood must be collected.
- Vitamin B_1 is light- and temperature sensitive; therefore the samples must be protected from light and refrigerated at 2-8° C.
- Samples are stable in the dark at 2-8° C for 1 day. For longer storage, samples should be frozen at -20 °C. **Do not** freeze and thaw repeatedly**.**

8.1 Sample pretreatment

Add 4 ml sample preparation buffer [SOL] to the bottle with the lyophilized enzyme [ENZ], close it and vortex.
Add 100 µl whole blood or control [CTRL1, CTRL2] to 400 µl of the prepared enzyme solution, mix and incubate at 37° C for 30 min in the dark. Afterwards, heat to 95 °C for 30 min, then cool quickly and centrifuge for 10 min at 10000 x g.

8.2 Sample dilution

Take 200 µl from the supernatant of the treated sample or control [CTRL1, CTRL2], add 200 µl water [DIL] and mix. The treatment and dilution results in a final dilution of 1:10 (= 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 from the kit the reagents and materials needed for the test. Put unused test kit components back into the refrigerator. Bring all necessary reagents to room temperature.

Water [DIL] for medium [ASYMED], standard [STD] and control [CRTL1, 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 an 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 95 °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).

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 QS test 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_1 [µg / l]	Water [DIL] [µl]	+	Standard [STD] [µl]	=	Total volume [µl]
Blank: 0	850	+	0	=	850
Standard 1: 3	850	+	150	=	1000
Standard 2: 6	700	+	300	=	1000
Standard 3: 9	370	+	300	=	670
Standard 4: 12	200	+	300	=	500
Standard 5: 15	200	+	600	=	800

Control [CTRL1, CTRL2]

- The controls must be freshly prepared before the test.
- Open the bottle of control [CTRL1, CTRL2], remove seal. Dispose of screw-top lid and seal.
- Add 0.5 ml water [DIL] from the test kit to the control bottle [CTRL1, CTRL2], close the bottle and dissolve by vortexing the bottle (= control 1, control 2).
- Treat the control afterwards as the sample is treated.
- Pipette 150 µl of the diluted control [CTRL1, CTRL2] into each well. We recommend to run a duplicate.
- For the concentration of the controls [CTRL1, CTRL2] please see control specification.

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 B_1 assay medium [ASYMED] in the cavities.
- Add 150 µl of standard [STD], respectively, sample or control [CTRL1, CTRL2] in the cavities. Pre-rinse the pipette tip with standard 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 h 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 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 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.

Vitamin B_1 in µg/l = Value from the standard curve x sample dilution factor (10)

11. EXPECTED VALUES

Vitamin B_1 concentration was determined in 42 samples of blood donors. The median value of this set of numbers was 48.1 (44.3) µg/ L. The array of 2-SD was 30 - 66 µg/ L. Figure 1 shows the distribution of the blood donor´s values.

Distribution of concentrations

Numer of samples	42
Mean	48.1
Median	44.3
SD	8.9
MW-2* SD	30.2
MW+2*SD	65.9

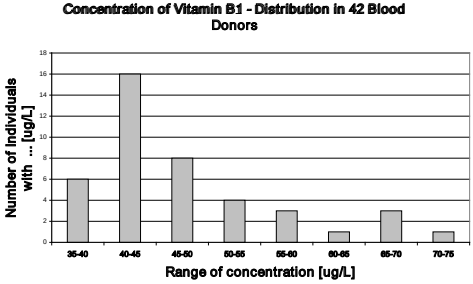


Fig. 1: Distribution of blood donor values

Please note: A concentration range of 30-150 µg/l Vitamin B_1 is covered at a sample dilution 1 : 10. 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.

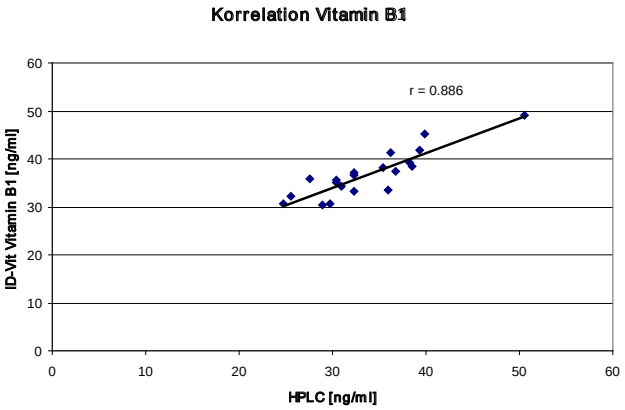
12. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n = 28)		
	Vitamin B_1 [µg/l] Mean value	VK [%]
Sample 1	54.5	2.75
Inter-Assay (n = 5)		
	Vitamin B_1 [µg/l] Mean value	VK [%]
Sample 1	56.94	3.81

Correlation to HPLC

The concentration of vitamin B_1 was determined by the ID-Vit®Vitamin B_1 assay in parallel to HPLC in 21 samples. Correlation coefficient: r = 0.886. Regression line: y = 0,7215x + 12,376.



13. REFERENCES

Koike H et al. (2006) Myopathy in thiamine deficiency: Analysis of a case. J Neurol Sci Aug 18
Lonsdale D (2006) A review of the biochemistry, metabolism and clinical benefits of thiamin(e) and its derivatives. Evid Based Complement Alternat Med. 2006 Mar;3(1):49-59

14. 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.

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