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Manual

ID-Vit® vitamin B_1

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

For use in human and veterinary medicine and in research

Valid from 2019-05-22

REF **KIF001**

Σ 96

+8°C +2°C

IVD CE

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1. INTENDED USE

ID-Vit® Vitamin B_1 is a microtiter plate test kit based on a microbiological method which measures the total vitamin B_1 content in whole blood. 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

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 beriberi, characterised 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. Myopathy also was found in relation to thiamine deficiency.

Indications for vitamin B_1 determination

- Suspicion of vitamin B_1 deficiency
- Determination of the metabolically 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 suspected neuritis

3. PRINCIPLE OF THE TEST

The whole blood samples are pre-treated and diluted with a buffer mixture, and then transferred into the wells of a microtiter plate coated with *Lactobacillus fermentum*. The addition of vitamin B_1 in either standards 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 compared to a standard curve generated from the dilution series. The amount of vitamin B_1 is directly proportional to the turbidity.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KIF001	PLATE	*Lactobacillus fermentum*-precoated microtiter plate	1 x
KIF001	SOL	Sample preparation buffer	5 x 5 ml
KIF001	ENZ	Enzyme, lyoph.	5 x
KIF001	DIL	Water	4 x 30 ml
KIF001	ASYMED	Vitamin B_1 assay medium	4 x
KIF001	STD	Vitamin B_1 standard, lyoph.	4 x
KIF001	FOL	Adhesive cover foil	4 x
KIF001	FRA	Replacement holder for microtiter strips	1 x
KIF001	CTRL1	Vitamin B_1 control 1, lyoph.	4 x
KIF001	CTRL2	Vitamin B_1 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)
- Water bath or thermoblock with variable temperatur (37 °C, 95 °C)
- ELISA reader 610–630 nm (540–550 nm)
- Calibrated precision pipettors and 20–1000 µl tips
- 5 ml and 10 ml pipets
- 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 value measured, 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 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 lid up and pull it back to the rim of the glass, then twist the whole cap off.

7.2 Preparation of the enzyme solution

- Add 4 ml sample preparation buffer [SOL] to a vial of lyophilised enzyme [ENZ], then homogenise using a vortex.
- Enzyme solution cannot be stored.

7.3 Preparation of the controls

- The lyophilised controls [CTRL1, CTRL2] have to be resuspended with each 500 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.
- 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_1 [µg/l]	**Water [DIL] [µl]**	**+**	**Standard concentrate [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

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 sterile 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 whole blood 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.

8.1 Sample pretreatment

Add 100 µl whole blood/control to 400 µl of prepared enzyme solution (ratio 1:5), mix, and incubate at 37 °C for 30 min in the dark. Then heat to 95 °C for 30 min, cool quickly (at 2–8 °C for 10 min) and centrifuge for 10 min at 10 000 *g*.

8.2 Sample dilution

Take 200 µl from the supernatant of the prepared serum/control, add 200 µl water [DIL] and mix. The sample treatment and dilution result in a total dilution of 1:10 (= 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 E 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 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_1 in µg/l = value from the standard curve × sample dilution factor (10)

Reference value for whole blood

Based on studies of blood samples of apparently healthy persons (n = 42), the following values were estimated.

Vitamin B_1: 30–66 µg/l

Distribution

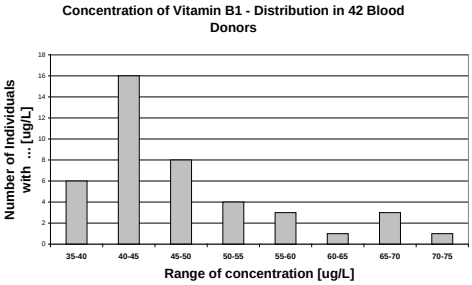


Fig. 1: Distribution of blood donor values

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

Please note

A concentration range of 30–150 µg/l vitamin B_1 is covered at a sample dilution of 1:10.

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

Serum/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 = 28)

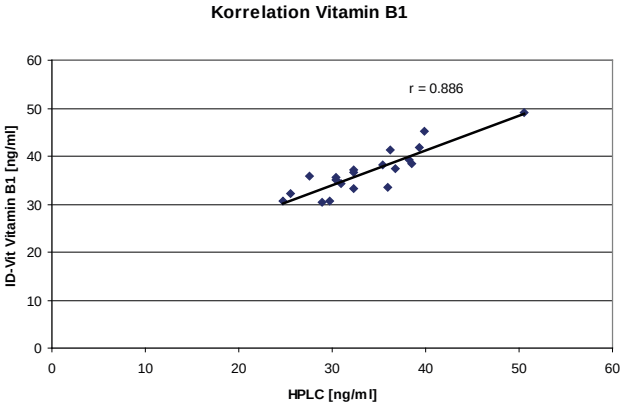
	Vitamin B_1 [µg/l]	**CV [%]**
Sample	54.5	2.75

Interassay (n = 5)

	Vitamin B_1 [µg/l]	CV [%]
Sample	56.94	3.81

12.2 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

1. Koike, H. et al., 2006. Myopathy in thiamine deficiency: analysis of a case. *Journal of the neurological sciences*, **249**(2), pp.175–9.
2. Lonsdale, D., 2006. A review of the biochemistry, metabolism and clinical benefits of thiamin(e) and its derivatives. *Evidence-based complementary and alternative medicine : eCAM*, **3**(1), pp.49–59.
3. Dzed, L. et al., 2015. Status of Thiamin deficiency in boarding school children from seven districts in Bhutan with previous history of peripheral neuropathy outbreaks : a cohort study. *Bhutan Health Journal*, **1**(1), pp.49–56.

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: