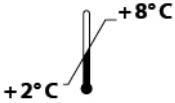
ID-Vit® *Vitamin B_6 Assay Kit*

Microbiological test kit for the determination of vitamin B_6 in serum with a Saccharomyces cerevisiae coated microtitre plate

Gültig ab / Valid from 13.03.2012

REF KIF006



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1. INTENDED USE

ID-Vit®*Vitamin B_6* is a microtiter plate test kit based on a microbiological assay which measures the vitamin B_6 content (pyridoxine, pyridoxal, pyridoxamine) 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 B_6 content. For use in human and veterinary medicine and in research. The test kit is For Research Use only.

2. INTRODUCTION

The vitamin B_6 group comprises three natural forms: pyridoxine, pyridoxamine and pyridoxal, which, during metabolism, are converted to the enzymatically active form pyridoxal-5-phosphate (P5P). Pyridoxal-5-phosphate (here described as „vitamin B_6 “) is a cofactor in more than a hundred enzyme reactions. One of its functions is to perform transamination, a key step in both breaking down and producing amino acids in the body. Vitamin B_6 is also necessary for the synthesis of neurotransmitters, as well as hemoglobin in red blood cells. And vitamin B_6 plays a central role in fat metabolism.

Vitamin B_6 deficiency symptomes

- Disturbance of protein biosynthesis
- Muscular weakness, loss of muscle control
- Skin disorders (dermatitis, pigment abnormality)
- Nervous disorders (irritability, depression, palsy)
- Insomnia

Vitamin B_6 deficiency can be considered as a risk factor for myocardial infarction, peripheral vascular deseases and atherosclerosis, especially in connection with the regulation of homocysteine metabolism.

Indications

Vitamin B_6 deficiency can result from:

- Chronic inflammatory bowel diseases (colitis ulcerosa, Crohn´s disease, gluten sensitivity)
- Dialysis
- Alcohol abuse
- Homocysteinuria
- Pregnancy and lactation

3. PRINCIPLE OF THE TEST

The serum samples are enzymatically pre-treated in order to determine the total vitamin B_6 content. The diluted samples are transferred in the wells of a microtiter plate [PLATE] coated with ***Saccharomyces cerevisiae*** which metabolizes vitamin B_6. The addition of vitamin B_6 in either standards [STD], controls [CTRL] or samples gives a vitamin B_6-dependent growth response until vitamin B_6 is consumed. After incubation at **30°C** for **44 - 48 h**, the growth of ***Saccharomyces cerevisiae*** 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_6 is directly proportional to the turbidity.

4. MATERIAL SUPPLIED

Catalog No	Label	Kit Components	Quantity
KIF006MTP	PLATE	One ***Saccharomyces cerevisiae***-precoated microtiter plate, ready to use	12 x 8 wells
KIF006SO	SOL	Sample preparation buffer 5 ml, ready to use	4 x
KIF006ENZ	ENZ	Enzyme	4 x
KIF006DI	DIL	Water 30 ml	4 x
KIF006ME	ASYMED	Vitamin B_6 assay medium	4 x
KIF006ST	STD	Vitamin B_6 standard	4 x
KIF006KO1	CTRL1	Control 1	4 x
KIF006KO2	CTRL2	Control 2	4 x
KIF006FO	FOL	Cover plastic foil	4 x
KIF006FR	FRA	Replacement holder for 96-well plates	1 x

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Incubator with a dark incubation chamber, 30°C
- Water bath or thermo block, 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) 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 curve [STD] as well as a sample and controls [CTRL1, CTRL2] analysis.
- If a higher dilution results in a higher measured value, inhibitors like antimycotics might be present.
- Reagents should not be used beyond the expiration date shown on kit label.
- Wear protective 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 (A630nm > 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 vortexed and then centrifuged (5 min at 10000 g) prior to measurement and the resulting supernatant used in the test.

8.1. Sample preparation

- Resuspend the enzyme [ENZ] with sample preparation buffer [SOL]: add 4 ml sample preparation buffer [SOL] in in the flask containing the lyophilized enzyme [ENZ], close and vortex.

- Add 300 µl serum or control [CTRL1, CTRL2] to 300 µl of the prepared enzyme solution, vortex and incubate for 30 min at 37 °C in the dark. Afterwards, heat to 95°C for 30 min, cool quickly and centrifuge for 10 min at 10 000 x g.
- Take 100 µl from the supernatant of the treated serum sample, add 400 µl water [DIL] and mix. The sample 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 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 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).

Controls [CTRL1, CTRL2]

- The controls must be freshly prepared before each test.
- Open the bottle of control [CTRL1, CTRL2], place the screw-top lid upside-down on the work bench.
- Add 0,75 ml water [DIL] from the test kit to the control bottle [CTRL1, CTRL2], close the bottle and dissolve by vortexing the bottle (= control).
- Add 300 µl serum to 300 µl of the prepared enzyme solution, shake and incubate for 30 min at 37 °C in the dark. Afterwards, heat to 95°C for 30 min, cool quickly and centrifuge for 10 min at 10 000 x g.
- Take 100 µl from the supernatant of the treated control, add 400 µl water [DIL] and mix. The control treatment and dilution results in a final dilution of 1:10 (= dilution factor).
- 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 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_6 [µg / l]		Water [DIL] [µl]	+	Standard [STD] [µl]	=	Total volume [µl]
Blank:	**0**	940	+	0	=	940
Standard 1:	**0.36**	940	+	60	=	1000
Standard 2:	**1.2**	400	+	100	=	500
Standard 3:	**1.8**	350	+	150	=	500
Standard 4:	**2.4**	300	+	200	=	500
Standard 5:	**3.6**	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 B_6 assay medium [ASYMED] in the cavities.
- Add 150 µl standard [STD], sample and control [CTRL1, CTRL2], respectively, in the cavities. Pre-rinse the pipette tip with standard and sample solution respectively.
- Carefully seal the cavities with plastic foil [FOL]. Important: the cavities must be made airtight by pressing down with the hand!
- Keep at **30 °C** for 44 - 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 over again and carefully remove the foil [FOL], beginning with the upper, right corner and pulling diagonally backwards at an angle of 180°.
- 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 hrs incubation time, the microtiter plate may be stored for a maximum of 48 hrs in the refrigerator before measuring the turbidity.
- To prevent time-loss through holidays or weekends, the microtiter 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_6 in serum:
Vitamin B_6 [µg/l] = Value from the standard curve x sample dilution factor (10)
Please note:
- A concentration range of 3.6 - 36.1 µg/l vitamin B_6 is covered using a sample dilution factor of 10.
- If the enzymatic treatment is omitted, the phosphorylated and nonphosphorylated forms can be differentiated.

Reference value for human serum
(n=74) Vitamin B_6: 4.8 - 17.7 µg/l

We recommend each laboratory to develop its own normal range. The values mentioned above are only for orientation and can deviate from other published data.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n=16)		
	Vitamin B_6 [µg/l]	CV [%]
Sample 1	9.87	9.17
Inter-Assay (n=5)		
	Vitamin B_6 [µg/l]	CV [%]
Sample 1	10.98	6.78

Recovery

Samples from 3 patients were spiked with Vitamin B_6 and analyzed. The mean values are shown below:

n = 5

Sample	Mean value measured in original sample [µg/l]	Spike [µg/l]	Vitamin B_6 expected [µg/l]	Vitamin B_6 measured [µg/l]	Recovery Rate [%]
A	11.99	10	21.99	26.00	140
		20	31.99	35.60	118
				Recovery rate in total [%]	**129**

n = 5

Sample	Mean value measured in original sample [µg/l]	Spike [µg/l]	Vitamin B_6 expected [µg/l]	Vitamin B_6 measured [µg/l]	Recovery Rate [%]
B	9.46	10	19.46	22.99	135
		20	29.46	32.50	115
				Recovery rate in total [%]	**125**

n = 5

Sample	Mean value measured in original sample [µg/l]	Spike [µg/l]	Vitamin B_6 expected [µg/l]	Vitamin B_6 measured [µg/l]	Recovery Rate [%]
C	8.855	10	18.85	18.31	95
		20	28.85	32.36	118
				Recovery rate in total [%]	**107**

12. REFERENCES

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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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