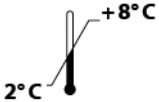
Manual
Preliminary

ID-Vit®*Vitamin B_2 Assay Kit in EDTA whole blood*

Microbiological test kit for the determination of vitamin B_2 in EDTA whole blood using a Lactobacillus rhamnosus coated microtitre plate

Valid from 13.03.2012

REF **KIF002VB**



ifp Institut für Produktqualität GmbH
Teltowkanalstr. 2
12247 Berlin, Germany
www.produktqualitaet.com

Distributed by:
Immundiagnostik AG
Stubenwald-Allee 8a
64625 Bensheim, Germany
www.immundiagnostik.com

1. INTENDED USE

ID-Vit®*Vitamin B_2* is a microtiter plate test kit based on a microbiological assay which measures the total Vitamin B_2 content in EDTA 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_2 content. For use in human and veterinary medicine and in research. The Vitamin B_2 Assay Kit is for research use only.

2. INTRODUCTION

Vitamin B_2 is a component of the flavin-nucleotides and as such is involved in the transport of hydrogen ions and electrons. It is an essential coenzyme for the metabolism of carbohydrates, fats and proteins.
The biological form of vitamin B_2 is riboflavin-5-phosphate. The most important vitamin B_2 derivatives are flavin mononucleotide (FMN) and flavin-adenine dinucleotide (FAD). In blood, about 60% of FAD and FMN are protein bound; only about 0.5 – 2 % occur in free form. Riboflavin-5-phosphate, FMN, and FAD are transported in the plasma by a variety of proteins, including albumin, fibrinogen, riboflavin-binding protein and other globulins. Although vitamin B_2 is eliminated regularly in the urine, its determination in urine samples is not recommended because of fluctuations in the concentration.

Indications for Vitamin-B_2-determination:

- Chronic diarrhea
- Preeclampsia
- Hypothyroidism
- Diabetes mellitus
- Alcohol abuse
- Anorexia
- Lactose intolerance

3. PRINCIPLE OF THE TEST

Pretreated EDTA whole blood samples are transferred in the wells of a microtiter plate [PLATE] coated with ***Lactobacillus rhamnosus***. The addition of Vitamin B_2 in either standards [STD] or samples gives a Vitamin B_2-dependent growth response until Vitamin B_2 is consumed. After incubation at **37°C** for **48 h**, the growth of ***Lactobacillus rhamnosus*** 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_2 is directly proportional to the turbidity.

4. MATERIAL SUPPLIED

Catalog No	Label	Kit Components	Quantity
KIF002VBMTP	PLATE	One ***Lactobacillus rhamnosus***-precoated microtiter plate, ready to use	12 x 8 wells
KIF002VBDI	DIL	Water 30 ml	4 x
KIF002VBME	ASYMED	Vitamin B_2 assay medium	4 x
KIF002VBST	STD	Vitamin B_2 standard	4 x
KIF002VBKO	CTRL	Vitamin B_2 control	4 x
KIF002VBFO	FOL	Cover plastic foil	4 x
KIF002VBFR	FRA	Replacement holder for 96-well plates	1 x
KIF002VBSO	SOL	Sample dilution buffer, ready to use	5 x 5 ml

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) 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] 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 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 Extinktion units (A_{630}nm > 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. Avoid repeated freezing and thawing.
- 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 100 µl of sample dilution buffer [SOL] to 100 µl of sample or control [CTRL], mix, heat at 95 °C for 15 min, rapidly cool down and centrifuge at 10000 x g for 10 min.

8.2. Sample dilution

Take 40 µl of the supernatant of the pretreated sample, add 360 µl of [SOL] and mix. The sample pretreatment and dilution corresponds to a total dilution of 1:20 (= 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 refridgerator. Bring all necessary reagents to room temperature.

Water [DIL] for medium [ASYMED], control [CTRL] and standard [STD]

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 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).

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_2 [µg / l]		Water [DIL] [µl]	+	Standard [STD] [µl]	=	Total volume [µl]
Blank:	**0**	900	+	0	=	900
Standard 1:	**5**	950	+	50	=	1000
Standard 2:	**10**	900	+	100	=	1000
Standard 3:	**20**	400	+	100	=	500
Standard 4:	**30**	350	+	150	=	500
Standard 5:	**60**	200	+	300	=	500

Control [CTRL]

- The control must be freshly prepared before the test.
- Open the bottle of control [CTRL], remove seal. Dispose of screw-top lid and seal.
- Add 5 ml water [DIL] from the test kit to the control bottle [CTRL], close the bottle and

dissolve by vortexing the bottle (= control).
- Treat the control afterwards as the sample is treated.
- Pipette 150 ml of the diluted control [CTRL] into each well. We recommend to run a duplicate.
- For the concentration of the control [CTRL] 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_2 assay medium [ASYMED] in the cavities.
- Add 150 µl standard [STD], control [CTRL], 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 lower, left 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 h 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.

EDTA whole blood

Vitamin B_2 in µg/l = Value from the standard curve x sample dilution factor (20)

Reference Value

Vitamin B_2 in whole blood: 126 - 1089 µg/l (n=40)

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		
	Vitamin B_2 [µg/l]	CV [%]
Sample 1 (n = 10)	12.21	4.47
Sample 2 (n = 6)	14.28	7.98
Inter-Assay		
	Vitamin B_2 [µg/l]	CV [%]
Sample 1(n = 10)	13.87	11.57
Sample 2 (n = 6)	11.30	10.69

12. REFERENCES

Powers HJ (2003) Riboflavin (vitamin B-2) and health. Am J Clin Nutr 77(6):1352-60. Review

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Test 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

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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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EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com