Manual

For professional use only

ID-Vit® vitamin B_2 in serum

Microbiological test kit for the determination of vitamin B_2 in serum using a Lactobacillus rhamnosus coated microtiter plate

For use in human and veterinary medicine and in research

Valid from 2025-03-28

REF **KIF002S** Σ 96

REF **KIF002S.2** Σ 2 x 96

+2 °C ±8 °C

IVD

CE

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Safety information

These accessories are to be used exclusively in accordance with the enclosed instructions for use. Important safety information for this product can be found in chapter 6.

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1. INTENDED PURPOSE

ID-Vit® Vitamin B_2 is a microtiter plate test kit based on a microbiological method which measures the vitamin B_2 content in serum. The test kit contains the standard and all reagents required to perform the test. 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

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 meta-bolism 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 diarrhoe
- Preeclampsia
- Hypothyroidism
- Diabetes mellitus
- Alcohol abuse
- Anorexia
- Lactose intolerance

3. PRINCIPLE OF THE TEST

The serum samples are pre-treated and diluted with a buffer mixture, and then transferred into the wells of a microtiter plate coated with *Lactobacillus rhamnosus*. The addition of vitamin B_2 in either standards or samples gives a vitamin B_2-dependent growth response until vitamin B_2 is consumed.

After incubation at **37 °C** for **72–77 h**, the growth of *Lactobacillus rhamnosus* is measured turbidimetrically at 610–630 nm (alternatively at 540–550 nm) in a microtiter plate reader and compared to a standard curve generated from the dilution series. The amount of vitamin B_2 is directly proportional to the turbidity.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity	
			KIF002S	KIF002S.2
KIF000.30	DIL	Water	4 x 30 ml	4 x 30 ml
KIF002S/ KIF002S.2	PLATE	*Lactobacillus rhamnosus*-precoated microtiter plate	1 x	2 x
	ASYMED	Vitamin B_2 assay medium	4 x	4 x
	STD	Vitamin B_2 standard, lyoph.	4 x	3 x
	FOL	Adhesive cover foil	1 x whole 3 x whole	3 x whole
	FRA	Replacement holder for microtiter strips	1 x	1 x
	CTRL1	Vitamin B_2 control 1, lyoph.	4 x	3 x
	CTRL2	Vitamin B_2 control 2, lyoph.	4 x	3 x

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Incubator with a dark incubation chamber, 37 °C
- ELISA reader 610–630 nm (540–550 nm)
- Calibrated precision pipettors and sterile single use 20–1 000 µl tips
- 5 ml and 10 ml pipets
- 1.5–2 ml reaction vials
- 0.2 µm sterile polyethersulfone (PES) filter with a disposable syringe (10 ml)
- 15 ml centrifuge tubes (e. g. Falcon tubes)
- Biocentrifuge (10 000 *g*)
- Vortex

6. PRECAUTIONS

- The test is based on a microbiological method. Contaminations lead to erroneous results.
- Water quality is extremely important for the test. Use only the water delivered with the test kit (**DIL**).
- For sterile filtration, only a sterile polyethersulfone filter must be used.
- It is essential to run a standard curve for each separate assay.
- Measure controls with each assay.
- We recommend measurements in duplicate.
- Do not use reagents beyond the expiration date shown on the label.
- As a precaution, it is recommended that the human material used is always considered potentially infectious.
- Used microtiter stripes and materials that have been in contact with patient samples must be handled and disposed of 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.
- 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 3 x (KIF002S.2) or 4 x (KIF002S) within the expiry date stated on the label.

7.1 Water

- Water (**DIL**) for medium (**ASYMED**), standard (**STD**) , controls (**CTRL1**, **CTRL2**) and dilutions.
- 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 sterile assay medium

- Fresh sterile assay medium has to be prepared each time before performing a test.
- Remove the desiccant bag from the lyophilised 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 vortex well. This amount is sufficient for 6 microtiter stripes.
- Filter the medium using a disposable syringe (10 ml) and the 0.2 µm PES filter into a centrifuge tube (15 ml, e. g. Falcon).
- After this preparation, the sterile assay medium can be used in the test.

Note: Any suspended solids present in the assay medium, which are removed by the sterile filtration, have no impact on the measured values.

7.3 Preparation of the controls

- The lyophilised controls (**CTRL1**, **CTRL2**) have to be resuspended each with **x ml** water (**DIL**) (x = see product specification) 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 water (**DIL**) (x = see quality control protocol) 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_2 [µg/l]	Water (DIL) [µl]	+	Standard concentrate [µl]	=	Total volume [µl]
Blank: 0	900	+	0	=	900
Standard 1: 25	975	+	25	=	1 000
Standard 2: 100	900	+	100	=	1 000
Standard 3: 200	400	+	100	=	500
Standard 4: 300	350	+	150	=	500
Standard 5: 600	200	+	300	=	500

7.5 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 serum for analysis.
- Samples are stable at 2–8 °C for one day in the dark. For longer storage, samples can be frozen and kept at -20 °C for up to 5 months.
- Centrifuge samples prior to measurement (at least 5 min at 10 000 *g*). Use the resulting supernatant in the test.
- Do not use hemolytic samples for analysis as they may give erroneous results. Centrifuge lipemic samples at 13 000 *g* for 10 min before assaying to obtain a serum that is as fat free as possible.

8.1 Sample dilution

Take 200 µl sample/control, add 200 µl water (**DIL**) and mix. The sample treatment and dilution result in a total dilution of 1:2 (= sample dilution factor).

9. ASSAY PROCEDURE

9.1 Test preparations

Take as many microtiter strips as needed from the kit. Return unused strips and any unused test kit components to the original packaging, and store 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 each cavity.
- Add 150 µl of the prepared standard dilutions (blank, standard 1–5), 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 **72–77 h** in an incubator.

9.3 Measurement

- Press the adhesive cover foil (**FOL**) firmly down again with the hand.
- Turn the microtiter plate (**PLATE**) upside down, place 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 72–77 h incubation time, the microtiter plate (**PLATE**) may be stored for a maximum of 48 h in the refrigerator before measuring the turbidity.

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 serves as a visual control to exclude contamination and is not taken into account in the calculation. The optical density must be < standard 1. If this is not the case, the analysis must be carried out again.

10.1 Calculation

Vitamin B_2 in µg/l = value from the standard curve × sample dilution factor (2).

Reference value for human serum

Based on studies of serum samples of apparently healthy persons (n = 40), the following values were estimated.

Vitamin B_2: 50–206 µg/l

Please note

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

Only serum can be used for the test.

12. PERFORMANCE CHARACTERISTICS

The following performance characteristics have been collected using human serum samples.

12.1 Precision and reproducibility

Intraassay

	Vitamin B_2 [µg/l]	CV [%]
Sample 1 (n=10)	12.21	4.47
Sample 2 (n=6)	14.28	7.98

Interassay

	Vitamin B_2 [µg/l]	CV [%]
Sample 1 (n=10)	13.87	11.57
Sample 2 (n=6)	11.30	10.69

13. 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.
- Do not use reagents beyond the expiration date stated on the kit label.
- Do not interchange different lot numbers of any kit component within the same assay.

- Follow the guidelines for medical laboratories.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which has not been consulted 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 made within 14 days after reception of the product. The product should be sent to Immundiagnostik AG along with a written complaint.
- Analyse controls with each run.
- Always perform assay according to the enclosed manual.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.

14. REFERENCES

Powers, H.J., 2003. Riboflavin (vitamin B-2) and health. *The American journal of clinical nutrition*, **77**(6), pp.1352–60.

15. SYMBOLS


Temperature limitation

In Vitro Diagnostic Medical Device


Manufacturer

Lot number


Contains plasma derivatives or human blood

Consult specification data sheet

Unique Device Identification


Medicinal substance

Catalogue number

To be used with

Content sufficient for <n> tests

Use by


Consult instructions for use


Do not re-use

Contains material of animal origin

Contains material of human origin

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