

ID-Vit[®] Vitamin B₂ aus Serum

*Microbiological test kit for the determination of
vitamin B₂ in serum using a
Lactobacillus rhamnosus coated microtitre plate
For use in human and veterinary research*

Valid from 2020-09-14



KIFR002S



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1. INTENDED USE

ID-Vit® Vitamin B₂ is a microtiter plate test kit based on a microbiological method which measures the vitamin B₂ content in serum. 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 research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Vitamin B₂ 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 B₂ is riboflavin-5-phosphate. The most important vitamin B₂ 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 B₂ is eliminated regularly in the urine, its determination in urine samples is not recommended because of fluctuations in the concentration.

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 B₂ in either standards or samples gives a vitamin B₂-dependent growth response until vitamin B₂ 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 compared to a standard curve generated from the dilution series. The amount of vitamin B₂ is directly proportional to the turbidity.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KIFR002S	PLATE	<i>Lactobacillus rhamnosus</i> -precoated microtiter plate	1 x
KIFR002S	SOL	Sample dilution buffer	4 x 5 ml

Cat. No.	Label	Kit components	Quantity
KIFR002S	DIL	Water	4 x 30 ml
KIFR002S	ASYMED	Vitamin B ₂ assay medium	4 x
KIFR002S	STD	Vitamin B ₂ standard, lyoph.	4 x
KIFR002S	FOL	Adhesive cover foil	4 x
KIFR002S	FRA	Replacement holder for microtiter strips	1 x
KIFR002S	CTRL1	Vitamin B ₂ control 1, lyoph.	4 x
KIFR002S	CTRL2	Vitamin B ₂ control 2, lyoph.	4 x
KIF002S	ASYBUF	Medium treatment buffer	4 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)
- Calibrated precision pipettors and sterile 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 (10000g)

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 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 controls

- The lyophilised controls [CTRL1, CTRL2] have to be resuspended with each 5 ml 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.

7.3 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 ₂ [µg/l]	Water [DIL] [µl]	+	Standard concentrate [µl]	=	Total volume [µl]
Blank: 0	900	+	0	=	900
Standard 1: 25	975	+	25	=	1000
Standard 2: 100	900	+	100	=	1000
Standard 3: 200	400	+	100	=	500
Standard 4: 300	350	+	150	=	500
Standard 5: 600	200	+	300	=	500

7.4 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] and 1 ml medium treatment buffer [ASYBUF] 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.

7.5 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 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 g before assaying to obtain fat free serum as far as possible.
- Samples should be centrifuged (at least 5 min at 10 000 g) prior to measurement. Use the resulting supernatant in the test.

8.1 Sample dilution

Take 200 µl sample/control, add 200 µl sample dilution buffer [SOL] 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 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 B₂ in µg/l = value from the standard curve × sample dilution factor (2)

Reference value for human serum

We recommend each laboratory to develop its own normal range as normal ranges strongly depend on the choice of the subject collective.

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

Whole blood 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

	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. REFERENCES

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

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- All reagents in the kit package are for research 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 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 sent 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:

	Temperature limitation		Catalogue Number
	For research use only		To be used with
	Manufacturer		Contains sufficient for <n> tests
	Lot number		Use by
	Attention		Consult instructions for use
	Consult specification data sheet		