Manual

For professional use only

ID-Vit® Biotin

Microbiological test kit for the determination of biotin in serum using a Lactobacillus plantarum coated microtiter plate
For use in human and veterinary medicine and in research

Valid from 2025-04-01

REF **KIF007** Σ 96

REF **KIF007.2** 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® Biotin is a microtiter plate test kit based on a microbiological method which measures the Biotin 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

Biotin (vitamin H) is widespread in bacteria, fungi, higher plants and animal tissues. The major part of biotin in food is covalently bound to protein with only a small part being freely available. During digestion, biocytin (biotinyl-lysin) is released from the proteins and can, similarly to biotin, easily be taken up from the intestine. Biotin is then released by the enzyme biocytinase from biocytin in plasma and erythrocytes and is available as prosthetic group for several biotin-dependent enzymes.

The daily biotin requirement is difficult to estimate because a healthy intestinal flora endogenously synthetises biotin and thereby helps to satisfy this need. Recent findings suggest that adults need a daily intake of 100–200 µg biotin. Supplementation on a miligram scale leads to significant improvement regarding neuropathology and glucose metabolism of chronic hemodialysis patients.

Biotin deficiency

Symptoms of biotin deficiency are caused by e. g. damage to the intestinal flora or extreme diets (e. g. frequent consumption of raw eggs). Consequences of biotin deficiency include dermatitis, hair loss, anorexia, muscular hypotonia, depression and sexual dysfunction.

Indications for a determination of biotin

- Defects in enzymes (e. g. genetic deficiency of biotinidase)
- Short gut syndrome
- Altered intestinal flora
- Malnutrition

3. PRINCIPLE OF THE TEST

The serum samples are diluted and then added into the wells of a microtiter plate coated with *Lactobacillus plantarum*. The addition of biotin in either standards or samples gives a biotin-dependent growth response until biotin is consumed. After incubation at **37 °C** for **46–50 h**, the growth of *Lactobacillus plantarum* 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 biotin is directly proportional to the turbidity.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity	
			KIF007	KIF007.2
KIF000.30	DIL	Water	4 x 30 ml	8 x 30 ml
KIF007/ KIF007.2	PLATE	*Lactobacillus plantarum*-precoated microtiter plate	1 x	2 x
	ASYMED	Biotin assay medium	4 x	4 x
	STD	Biotin standard, lyoph.	4 x	3 x
	FOL	Adhesive cover foil	1 x whole 3 x half	3 x whole
	FRA	Replacement holder for microtiter strips	1 x	1 x
	CTRL1	Biotin control 1, lyoph.	4 x	3 x
	CTRL2	Biotin 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 (KIF007.2) or 4 x (KIF007) within the expiry date stated on the label.

7.1 Water

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

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 of the controls

- The lyophilised controls (**CTRL1**, **CTRL2**) have to be resuspended each with **x µl** 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:

Biotin [µg/l]	Water (DIL) [µl]	+	Standard concentrate [µl]	=	Total volume [µl]
Blank: 0	500	+	0	=	500
Standard 1: 0.02	450	+	50	=	500
Standard 2: 0.06	350	+	150	=	500
Standard 3: 0.10	250	+	250	=	500
Standard 4: 0.14	150	+	350	=	500
Standard 5: 0.18	50	+	450	=	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 3 days in the dark. For longer storage, samples can be frozen and kept at -20 °C for p 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 50 µl from each sample/control, add 950 µl water (**DIL**) and mix. The sample dilution results in a total dilution of 1:20 (= 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 **46–50 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 46–50 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

Biotin in µg/l = value from the standard curve × sample dilution factor (20)

Reference value for human serum

Based on studies of serum samples of apparently healthy persons (n = 84), the following biotin values were estimated.

<750 ng/l	biotin deficiency requiring treatment
750–1 250 ng/l	suboptimal biotin status
>1 250 ng/l	sufficient levels of biotin

Please note

A concentration range of 0.4–3.6 µg/l Biotin is covered at a sample dilution of 1:20.

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 (n = 5)

	Biotin [µg/l]	CV [%]
Sample	63.5	3.4

Interassay (n = 5)

	Biotin [µg/l]	CV [%]
Sample	64.3	2.9

12.2 Recovery

Samples from 4 patients were spiked with biotin and analysed. The mean values are shown below.

Sample (n=5)	Mean value original sample [µg/l]	Spike [µg/l]	Biotin expected [µg/l]	Biotin measured [µg/l]	Recovery Rate [%]
A	1.1	2	3.1	3.3	110
		4	5.1	5.2	103
		6	7.1	7.3	103
			Recovery rate in total [%]		**105**

Sample (n=5)	Mean value measured in original sample [µg/l]	Spike [µg/l]	Biotin expected [µg/l]	Biotin measured [µg/l]	Recovery Rate [%]
B	1.5	2	3.5	3.2	85
		4	5.5	4.7	80
		6	7.5	7.4	98
				Recovery rate in total [%]	**88**

Sample (n=5)	Mean value measured in original sample [µg/l]	Spike [µg/l]	Biotin expected [µg/l]	Biotin measured [µg/l]	Recovery Rate [%]
C	1.4	2	3.4	3.3	95
		4	5.4	5.6	105
		6	7.4	7.0	93
				Recovery rate in total [%]	**98**

Sample (n=5)	Mean value measured in original sample [µg/l]	Spike [µg/l]	Biotin expected [µg/l]	Biotin measured [µg/l]	Recovery Rate [%]
D	1.3	2	3.3	3.6	115
		4	5.3	5.7	110
		6	7.3	6.9	93
				Recovery rate in total [%]	**106**

12.3 Linearity

Two patient samples were diluted and analysed. The results are shown below:

Sample	Dilution	Biotin expected [µg/l]	Biotin measured [µg/l]
A	20	3.0	3.1
	40		3.5
	80		3.3
	120		2.9
	160		3.4
B	20	1.3	1.3
	40		1.3
	80		1.6

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

Burtis, C.A. & Ashwood, E.R. eds., Tietz Textbook of Clinical Chemistry 3rd ed., Saunders.

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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