Manual

For professional use only

ID-Vit® folic acid

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

For use in human and veterinary medicine and in research

Valid from 2024-11-28

REF **KIF005** Σ 96

REF **KIF005.2** Σ 2 x 96

+2 °C ±8 °C

IVD

CE

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Safety information

The assay has to be performed exclusively according to the instructions for use enclosed with the kit. Important safety information for this product can be found in the chapter WARNINGS AND PRECAUTIONS.

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1. INTENDED PURPOSE

ID-Vit® Folic acid is a microtiter plate test kit based on a microbiological method which measures the total folic acid 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

Folic acid, a water soluble, light and temperature sensitive vitamin of the B complex (vitamin B_9), is involved in all growth and development processes of the body. Folic acid is essential for the formation of red blood cells, for optimal functioning of the bone marrow and for healthy nerve activity. Moreover, folic acid is essential for cell division, therefore it is important in foetus development.

Although most plant and animal based foods contain folic acid, a deficiency of folic acid is the most widespread vitamin deficiency in Europe and North America. According to information from the German Nutritional Society (Deutschen Gesellschaft für Ernährung) only one in four Germans absorb sufficient folic acid – the result of one-sided nutritional habits with little fresh fruit and vegetables. But also age, disease and the influence of specific medications, e.g. cotrimoxazol, may lead to resorption disturbances and to an associated deficiency.

Lowered folic acid levels occur because of:

- a decreased supply (e.g. through alcoholism or folic acid antagonists),
- a disrupted resorption (e.g. in celiac disease, CED),
- an increased requirement (e.g. during pregnancy, in anaemic or cancerous diseases).

Symptoms of Deficiency

The first symptoms of deficiency are weariness, irritability, concentration problems and loss of appetite; further consequences are inflammation of the mucous membranes, anaemia and grievous neurological damage.

During pregnancy, when the folic acid requirements are doubled, a deficiency in folic acid may lead to premature birth and severe abnormalities. An optimal supplementation of folic acid during the pregnancy can reduce the risk of neural tube defects in the foetus by 85 %.

Because a deficiency of either vitamin B_{12} or folic acid may lead to megalobastic anaemia, the determination of both vitamins is important for the clinical picture so that the correct vitamin may be supplemented. Otherwise, in the case of vitamin B_{12} deficiency, treatment of megaloblastic anaemia with folic acid may lead to irreversible damage of the central nervous system.

Folic acid and arteriosclerosis

A folic acid deficiency is known to be the most common cause of hyperhomocysteinaemia. Meanwhile, the hyperhomocysteinaemia has been recognised as an independent factor in arteriosclerosis. Therefore, the determination of folic acid can be carried out within the framework of a coronary disease risk analysis. Beside of the influence of folic acid on the homocysteine levels, a further positive effect on the endothelial function in heart patients has been established – development of nitrate tolerance during continuous nitrate therapy, e.g. in such patients, an increased release of oxygen radicals occurs without folic acid supplementation (Verhaar et al. 2002).

Indications

- Hyperchrome, macrocytic anemia
- Long-term therapy with antiepileptic drugs or folic acid antagonists
- Long-term haemodialysis
- Multiple birth pregnancy/planned pregnancy
- Enhanced erythropoiesis
- Chronic liver diseases
- Hemoblastosis
- Psoriasis, dermatitis
- Stomatitis, glossitis
- Chronic alcohol abusus

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 folic acid in either standards or samples gives a folic acid-dependent growth response until folic acid is consumed. After incubation at **37 °C** for **46–50 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 folic acid is directly proportional to the turbidity.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity	
			KIF005	KIF005.2
KIF000.30	DIL	Water	4 x 30 ml	7 x 30 ml
KIF005 KIF005.2	PLATE	*Lactobacillus rhamnosus*-precoated microtiter plate	1 x	2 x
	ASYMED	Folic acid assay medium	4 x	4 x
	STD	Folic acid 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	Folic acid control 1, lyoph.	4 x	3 x
	CTRL2	Folic acid 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. WARNINGS AND 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 (KIF005.2) or 4 x (KIF005) 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

7.3 Preparation 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:

Folic acid [µg/l]		Water (DIL) [µl]	+	Standard concentrate [µl]	=	Total volume [µl]
Blank:	0	500	+	0	=	500
Standard 1:	0.04	450	+	50	=	500
Standard 2:	0.12	350	+	150	=	500
Standard 3:	0.20	250	+	250	=	500
Standard 4:	0.28	150	+	350	=	500
Standard 5:	0.36	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 8 hours 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 10 µl sample/control, add 740 µl water (**DIL**) and mix. The sample dilution result in a total dilution of 1:75 (= 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 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

Folic acid in µg/l = value from the standard curve × sample dilution factor (75).

Reference value for human serum

Based on studies of serum samples of apparently healthy persons (n = 74), the following values were estimated.

Folic acid: 3.8–23.2 µg/l

Please note

A concentration range of 3–27 µg/l folic acid is covered at a sample dilution of 1:75.

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 = 21)

	Folic acid [µg/l]	CV [%]
Sample	12.69	4.7

Interassay (n = 3)

	Folic acid [µg/l]	CV [%]
Sample	12.24	5.68

12.2 Recovery

Samples from 4 patients were differently diluted (75, 150, 300), spiked with folic acid and analysed. The mean values are shown below.

Sample (n=9)	Mean value original sample [µg/l]	Spike [µg/l]	Folic acid expected [µg/l]	Folic acid measured [µg/l]	Recovery Rate [%]
A	8.2	5	13.2	13.8	112
		10	18.2	19.1	109
		15	23.2	24.8	111
				Recovery rate in total [%]	**111**

Sample (n=8)	Mean value original sample [µg/l]	Spike [µg/l]	Folic acid expected [µg/l]	Folic acid measured [µg/l]	Recovery Rate [%]
B	3.9	5	8.9	9.3	108
		10	13.9	14.3	104
		15	18.9	19.5	104
				Recovery rate in total [%]	**105**

Sample (n=8)	Mean value original sample [µg/l]	Spike [µg/l]	Folic acid expected [µg/l]	Folic acid measured [µg/l]	Recovery Rate [%]
C	4.4	5	9.4	9.6	104
		10	14.4	14.5	101
		15	19.4	20.0	104
				Recovery rate in total [%]	**103**

Sample (n=8)	Mean value original sample [µg/l]	Spike [µg/l]	Folic acid expected [µg/l]	Folic acid measured [µg/l]	Recovery Rate [%]
D	5.1	5	10.1	10.6	110
		10	15.1	15.3	102
		15	20.1	20.6	103
				Recovery rate in total [%]	**105**

12.3 Linearity

Samples from 2 patients were diluted and analysed. The results are shown below.

Sample	Dilution	Folic acid expected [µg/l]	Folic acid detected [µg/l]
A	75	13.2	13.7
	150		14.0
	300		13.9
C	150	19.4	20.1
	300		20.7
	150		19.4

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. DISPOSAL

Liquid test components, microtiter plates and vials should be treated as ordinary laboratory waste unless otherwise stated. Specimens and other potentially infectious materials must be disposed of in accordance with regulatory requirements.

15. REFERENCES

1. Obeid, R. & Herrmann, W., 2006. Mechanisms of homocysteine neurotoxicity in neurodegenerative diseases with special reference to dementia. *FEBS letters*, **580**(13), pp.2994–3005.
2. Strohecker, R. & Henning, H., *1963*. Vitamin-Bestimmungen. Erprobte Methoden. E. Merck AG, ed., Weinheim/Bergstraße: Verlag Chemie GmbH.
3. Verhaar, M.C., Stroes, E. & Rabelink, T.J., 2002. Folates and cardiovascular disease. *Arteriosclerosis, thrombosis, and vascular biology*, **22**(1), pp.6–13.

16. 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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