

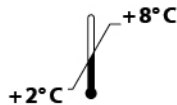
ID-Vit® *Folic Acid Assay Kit*

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

Valid from 13.03.2012



KIF005



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®Folic acid is a microtiter plate test kit based on a microbiological assay which measures the total folic acid content in serum. 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 folic acid content. For use in human and veterinary medicine and in research. The Folic Acid Assay kit is For Research Use Only.

2. INTRODUCTION

Folic acid, a water soluble, light and temperature sensitive vitamin of the B complex (vitamin B₉), 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 patients 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 B₁₂ or folic acid may lead to megaloblastic 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 B₁₂ 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

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

Reference:

Verhaar et al. (2002) Folates and cardiovascular diseases. *Arterioscler Thromb Vasc Biol* 22: 6-13

3. PRINCIPLE OF THE TEST

Serum samples are diluted with a buffer solution. The diluted samples are added into the microtiter plate wells [PLATE] coated with *Lactobacillus rhamnosus* which metabolizes folic acid. The addition of folic acid in either standards [STD] or samples gives a folic acid-dependent growth response until it is consumed. After incubation at **37°C** for **48 h**, the growth of *Lactobacillus rhamnosus* is measured turbidimetrically at 610 - 630 nm (alternative at 540 - 550 nm) in an ELISA-reader and a standard curve is generated from the dilution series. The amount of folic acid is directly proportional to the turbidity.

4. MATERIAL SUPPLIED

Catalog No	Label	Kit Components	Quantity
KIF005MTP	PLATE	One <i>Lactobacillus rhamnosus</i> precoated microtiter plate, ready to use	12 x 8 wells
KIF005SO	SOL	Sample treatment solution 5 ml, ready to use	4 x
KIF005DI	DIL	Water 30 ml, ready to use	4 x
KIF005ME	ASYMED	Folic acid-Assay-Medium	4 x
KIF005ST	STD	Folic acid-Standard	4 x
KIF005FO	FOL	Cover plastic foil	4 x
KIF005FR	FRA	Replacement holder for 96-well plates	1 x
KIF005BU	ASYBUF	Folic acid medium treatment buffer 1.5 ml	4 x
KIF005KO1	CTRL1	Control Folic acid 1	4 x
KIF005KO2	CTRL2	Control Folic acid 2	4 x

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- 1 Incubator with a dark incubation chamber, 37 °C
- 1 Water bath (90°C - 100°C)
- 1 ELISA-Reader 610 - 630 nm (540 - 550 nm)
- 1 Micropipette 20 - 200 µl
- 1 Micropipette 100 -1000 µl
- 1 Micropipette tips to deliver 20 - 200 µl and 100 -1000 µl, sterile
- 1 Pipettes of 5 and 10 ml
- 1 1.5 - 2 ml reaction vials, sterile
- 1 0.2 µm sterile polyethersulfone filter with a sterile tip
- 1 15 ml centrifugal tubes, sterile (e.g. Falcon tubes)
- 1 Biocentrifuge (10 000 x g)

6. PREPARATION AND STORAGE OF REAGENTS

- 1 Store test kit / reagents at 2-8°C.
- 1 Prepare reagents freshly and use immediately after preparation. Discard remaining unused reagents and waste in accordance with country, federal, state, and local regulations.
- 1 Put unused reagents (standard, medium) in the test kit and store at 2-8°C.
- 1 Store holder with unused strips in the original package with the dry bag at 2-8° C to prevent contamination or moisture exposure.
- 1 No warranty can be given after the expiry date (see label of test package).
- 1 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

- 1 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).
- 1 GLP (Good Laboratory Practice)-guidelines should be observed.
- 1 **Water quality** is extremely important. Only the water delivered with the test kit [DIL] should be used for medium dilution [ASYMED], standard [STD] and control [CTRL1, CTRL2] reconstitution as well as for sample preparation.
- 1 For sterile filtration, only a sterile polyethersulfone filter must be used.
- 1 It is essential to run a standard curve for each separate assay.
- 1 It is recommended to run a duplicate standard [STD] curve as well as a sample analysis.
- 1 If a higher dilution results in a higher measured value, inhibitors like antibiotics might be present.
- 1 Reagents should not be used beyond the expiration date shown on kit label.
- 1 Wear gloves during the test
- 1 Used microtiter plates [PLATE] and materials that have been in contact with patient's samples should be handled and disposed as potentially infectious.
- 1 Signs for reagent damage: The highest standard should have an absorption higher than 0.6 Extinction units ($A_{630\text{nm}} > 0.6$)

8. SAMPLE PREPARATION

Notes

- 1 Patient serum is used for analysis.
- 1 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 (6-8 weeks), samples should be frozen at -20°C (folic acid is light sensitive).
- 1 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.
- 1 Samples should be centrifuged (5 min at 10000 g) prior to measurement and the resulting supernatant used in the test.

8.1 Sample treatment/extraction

Add 100 µl of serum sample or controls [CTRL1, CTRL2] to 400 µl of sample preparation solution [SOL], heat to 95°C for 30 min and then cool fast. Afterwards, centrifuge (minimum 5 min at 10000 x g).

8.2 Sample dilution

Take 50 µl from the supernatant of the treated serum sample or control [CTRL1, CTRL2], add 700 µl of water [DIL] and mix (alternatively 25 µl serum and 350 µl water [DIL]). The sample treatment and dilution results in a final dilution of 1:75 (= sample dilution factor).

9. ASSAY PROCEDURE

Procedural notes

- 1 Quality control guidelines should be observed.
- 1 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.
- 1 The assay should always be performed according the enclosed manual.

9.1 Test preparations

Take as many microtiter strips as needed from kit. Put unused strips in the original package bag, and return the remaining parts of the test kit to the refrigerator. Bring all necessary reagents to room temperature.

Water [DIL] for medium [ASYMED] and standard [STD]

Push the lid up, pull it back to the rim of the glass and remove the entire seal by turning.

Assay medium [ASYMED]

- 1 The medium must be freshly prepared before each test.
- 1 Take the dry bag out of medium vial [ASYMED] by tweezers, shake off and discard.
- 1 Add 1 ml medium treatment buffer [ASYBUF] and 10 ml of water [DIL] to the assay medium [ASYMED], securely close the bottle and shake well. The amount is sufficient for 6 strips.
- 1 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 is firmly closed at all times.
- 1 Quickly cool the medium bottle to under 30 °C.
- 1 Filter 10 ml medium [ASYMED] sterilely with a 0.2 µm filter in a centrifuge test tube. (e.g. 15 ml, Falcon).

Standard [STD]

- 1 Before the test, freshly prepare the standard curve solutions:
- 1 Open the bottle of standard [STD], place the screw-top lid upside-down on the work bench.
- 1 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).
- 1 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:

Folic acid [µg / l]	Water [DIL] [µl]	+	Standard [STD] [µl]	=	Total volume [µl]
Blank: 0	450	+	0	=	450
Standard 1: 0.04	450	+	50	=	500
Standard 2: 0.08	400	+	100	=	500
Standard 3: 0.16	300	+	200	=	500
Standard 4: 0.24	200	+	300	=	500
Standard 5: 0.32	100	+	400	=	500

Controls [CTRL1, CTRL2]

- 1 The controls must be freshly prepared before the test.
- 1 Open the bottle of controls [CTRL1, CTRL2], remove seal. Dispose of screw-top lid and seal.
- 1 Add 0.125 ml of water [DIL] from the test kit to the control bottles [CTRL1, CTRL2], close the bottles and vortex (= control 1, control 2).
- 1 Treat the controls [CTRL1, CTRL2] afterwards as the sample is treated.
- 1 Pipette 150 µl of the pretreated and diluted controls [CTRL1, CTRL2] into each well. We recommend to run the controls [CTRL1, CTRL2] in duplicate.
- 1 For the concentration of the Controls [CTRL1, CTRL2] please see Control specification.

9.2 Test Initiation

- 1 Take as many microtiter strips as needed from the kit in put them in the second microtiter strip holder [FRA]. Store unused strips in the original package bag at 2-8° C to prevent contamination or moisture exposure.
- 1 A medium solution is sufficient for 6 strips. (= 48 cavities)
- 1 Put 150 µl Folic acid assay medium [ASYMED] in the cavities.
- 1 Add 150 µl standard [STD], control [CTRL1, CTRL2] or sample in the respective cavities. Pre-rinse the pipette tip with standard, control or sample solution respectively.
- 1 Carefully seal the cavities with plastic foil [FOL]. Important: the cavities must be made airtight by pressing down with the hand!
- 1 Keep at **37 °C** for 48 hrs in an incubator.

9.3 Measurement

- 1 Securely press the foil [FOL] down with the hand.
- 1 Upturn the plate [PLATE] onto a tabletop and shake the germination well.
- 1 Turn the plate [PLATE] over again and **carefully** remove the foil [FOL], beginning with the upper right corner and pulling diagonally backwards at an angle of 180°. During this fix the strips in the frame with your hand because the foil is highly adhesive.
- 1 Remove air bubbles in the cavities using a pipette tip or a needle.
- 1 Read turbidity in an ELISA-Reader at E610 - 630 nm (alternatively at 540 - 550 nm)

Please note

- 1 After 48 hrs incubation time, the microtiter platter may be stored for a maximum of 48 hrs in the refrigerator before measuring the turbidity.
- 1 To prevent time-loss through public holidays or weekends, the microtiter 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.

Serum

Folic acid in $\mu\text{g} / \text{l} = \text{Value from the standard curve} \times \text{dilution factor} (= 75)$

Reference value for human serum

Folic acid: 3.8 - 23.2 $\mu\text{g}/\text{L}$ (n=74)

Please note: A concentration range of 3 - 24 $\mu\text{g}/\text{L}$ folic acid is covered using a sample dilution factor of 75.

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 (n=19)		
Sample 1	Folic acid [$\mu\text{g}/\text{L}$]	VC[%]
	12.69	4.70
Inter-Assay (n =5)		
Sample 1	Folic acid [$\mu\text{g}/\text{L}$]	VC[%]
	12.24	5.68

Recovery

Samples from 4 patients were diluted differently, spiked with folic acid and analyzed. The mean values are shown below:

n = 9

Sample	Mean value measured in original sample [$\mu\text{g/L}$]	Spike [$\mu\text{g/L}$]	Folic acid expected [$\mu\text{g/L}$]	Folic acid detected [$\mu\text{g/L}$]	Recovery Rate [%]
A	8.2 $\mu\text{g/L}$	5	13.2	13.8	112
		10	18.2	19.1	109
		15	23.2	24.8	111
Recovery rate in total [%]					111

n = 8

Sample	Mean value measured in original sample [$\mu\text{g/L}$]	Spike [$\mu\text{g/L}$]	Folic acid expected [$\mu\text{g/L}$]	Folic acid detected [$\mu\text{g/L}$]	Recovery Rate [%]
B	3.9 $\mu\text{g/L}$	5	8.9	9.3	108
		10	13.9	14.3	104
		15	18.9	19.5	104
Recovery rate in total [%]					105

n = 8

Sample	Mean value measured in original sample [$\mu\text{g/L}$]	Spike [$\mu\text{g/L}$]	Folic acid expected [$\mu\text{g/L}$]	Folic acid detected [$\mu\text{g/L}$]	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

n = 8

Sample	Mean value measured in original sample [$\mu\text{g/L}$]	Spike [$\mu\text{g/L}$]	Folic acid expected [$\mu\text{g/L}$]	Folic acid detected [$\mu\text{g/L}$]	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

Linearity

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

n = 2

Sample	Dilution	Folic acid expected [$\mu\text{g/L}$]	Folic acid detected [$\mu\text{g/L}$]
A	75	13.2	13.7
	150		14.0
	300		13.9
C	150	19.4	20.1
	300		20.7
	450		19.4

12. REFERENCES

Verhaar et al. (2002) Foliates and cardiovascular diseases. *Arterioscler Thromb Vasc Biol* 22:6-13

Obeid R, Herrmann W (2006) Mechanism of homocysteine neurotoxicity in neurodegenerative diseases with special reference to dementia. *FEBS Lett* May 29;580(13):2994-3005

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- 1 Test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- 1 All reagents in the test package are for use in human and veterinary medicine and in research. For Research Use only.
- 1 Reagents should not be used after the date of expiry stated on the label.
- 1 Single components with different lot numbers should not be mixed or exchanged.
- 1 Guidelines for medical laboratories should be observed.
- 1 Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variation of the test procedure that is not coordinated with the producer may influence the results of the test.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

distributed in the US/Canada by:

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



EAGLE
BIOSCIENCES



Immundiagnostik AG

Stubenwald-Allee 8a

D-64625 Bensheim

Tel.: +49 (0) 62 51/70 19 00

Fax: +49 (0) 62 51/84 94 30

info@immundiagnostik.com

www.immundiagnostik.com