



## DRG<sup>®</sup> Vitamin Folic Acid (BIO-4886)



Revised 27 Aug. 2010 rm (Vers. 4.1)

*Please use only the valid version of the package insert provided with the kit.*

### INTENDED USE

*Vitamin Folic Acid Test* 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 Research Use Only.

### PRINCIPLE OF THE TEST

Serum samples are diluted with a buffer solution. The diluted samples are added into the microtiter plate wells [coated with *Lactobacillus rhamnosus* which metabolizes folic acid. The addition of folic acid in either standards 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 plantarum* 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.

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### MATERIAL SUPPLIED

	Kit Components	Quantity
<b>PLATE</b>	One <i>Lactobacillus rhamnosus</i> -precoated microtiter plate, ready to use	12 x 8 wells
<b>SOL</b>	Sample treatment solution 5 mL, ready to use	4 x
<b>DIL</b>	Water 30 mL, ready to use	4 x
<b>ASYMED</b>	Folic acid-Assay-Medium	4 x
<b>STD</b>	Folic acid-Standard	4 x
<b>FOL</b>	Cover plastic foil	4 x
<b>FRA</b>	Replacement holder for 96-well plates	1 x
<b>ASYBUF</b>	Folic acid medium treatment buffer 1.5 mL	4 x
<b>CTRL1</b>	Control Folic acid 1	4 x
<b>CTRL2</b>	Control Folic acid 2	4 x

### 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)
- Micropipette 20 - 200 µL
- Micropipette 100 -1000 µL
- Micropipette tips to deliver 20 - 200 µL and 100 -1000 µL, sterile Pipettes of 5 and 10 mL
- 1.5 - 2 mL reaction vials, sterile
- 0,2 µm sterile polyethersulfone filter with a sterile tip
- 15 mL centrifugal tubes, sterile (e.g. Falcon tubes)
- Biocentrifuge (10 000 x g)

### PREPARATION AND STORAGE OF REAGENTS

- Store test kit / reagents at 2-8°C.
- Prepare reagents freshly and use immediately after preparation. Discard remaining unused reagents and waste in accordance with country, federal, state, and local regulations.
- Put unused reagents (standard, medium) in the test kit and store at 2-8°C.
- Store holder with unused strips in the original package with the dry bag at 2-8° C to prevent contamination or moisture exposure.

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Revised 27 Aug. 2010 rm (Vers. 4.1)

- No warranty can be given after the expiry date (see label of test package).
- 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.

**PRECAUTIONS**

- 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).
- GLP (Good Laboratory Practice)-guidelines should be observed.
- **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.**
- It is essential to run a standard curve for each separate assay.
- It is recommended to run a duplicate Standard curve as well as a sample analysis.
- If a higher dilution results in a higher measured value, inhibitors like antibiotics might be present.
- Reagents should not be used beyond the expiration date shown on kit label.
- Wear gloves during the test
- Used microtiter plates and materials that have been in contact with specimen's samples should be handled and disposed as potentially infectious.
- Signs for reagent damage: The highest standard should have an absorption higher than 0.6 Extinction units ( $A_{630nm} > 0,6$ )

**SAMPLE PREPARATION****Notes**

- Sample serum is used for analysis.
- 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).
- 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.
- Samples should be centrifuged (5 min at 10000 g) prior to measurement and the resulting supernatant used in the test.

**Sample treatment/extraction**

Add 100 µL serum sample or controls [CTRL1, CTRL2] to 400 µL Sample Preparation Solution, heat to 95°C for 30 min and then cool fast. Afterwards, centrifuge (minimum 5 min at 10000 x g).

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**Sample dilution**

Take 50  $\mu$ L from the supernatant of the treated serum sample or controls [CTRL1, CTRL2], add 700  $\mu$ L water [*DIL*] and mix (alternatively 25  $\mu$ L serum and 350  $\mu$ L water *DIL*).

The sample treatment and dilution results in a final dilution of 1:75 (= sample dilution factor).

**ASSAY PROCEDURE**

***Procedural notes***

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

**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.

Revised 27 Aug. 2010 rm (Vers. 4.1)

**Assay medium [ASYMED]**

- The medium must be freshly prepared before each test.
- Take the dry bag out of medium vial [ASYMED] by tweezers, shake off and discard.
- 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.
- 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.
- Quickly cool the medium bottle to under 30 °C.
- Filter 10 mL medium [ASYMED] sterilely with a 0.2 µm filter in a centrifuge test tube. (e.g. 15 mL, Falcon).

**Standard**

Before the test, freshly prepare the standard curve solutions:

- Open the bottle of standard, place the screw-top lid upside-down on the work bench.
- Add x mL (x = see QS test kit data sheet) water [DIL] from the test kit to the standard bottle, close the bottle and shake (= standard concentrate).
- 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 [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**

- The controls must be freshly prepared before the test.
- Open the bottle of control [CTRL1, CTRL2], remove seal. Dispose of screw-top lid and seal.
- Add 0.125 mL water [DIL] from the test kit to the control bottle [CTRL1, CTRL2], close the bottle and dissolve by vortexing the bottles (= control1, control2).
- Treat the control afterwards as the sample is treated.
- Pipette 150 µL of the pretreated and diluted controls [CTRL1, CTRL2] into each well. We recommend to run a duplicate.
- For the concentration of the controls [CTRL1, CTRL2] please see Control specification.

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**Revised 27 Aug. 2010 rm (Vers. 4.1)**

**Test Initiation**

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

**Measurement**

- Securely press the foil down with the hand.
- Upturn the plate onto a tabletop and shake the germination well.
- Turn the plate over again and carefully remove the foil, 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.
- Remove air bubbles in the cavities using a pipette tip or a needle.
- Read turbidity in an ELISA-Reader at E 610 - 630 nm (alternatively at 540 - 550 nm)

**Please note**

- After 48 hrs incubation time, the microtiter platter may be stored for a maximum of 48 hrs in the refrigerator before measuring the turbidity.
- To prevent time-loss through public holidays or weekends, the microtiter plate may also be evaluated after 60 hrs incubation.

**REFERENCES**

Verhaar et al. (2002) Folates 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

**GENERAL NOTES ON THE TEST AND TEST PROCEDURE**

- Test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the test package are for use in human and veterinary medicine and in research. For Research Use Only.
- Reagents should not be used after the date of expiry stated on the label.

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- Single components with different lot numbers should not be mixed or exchanged.
- Guidelines for medical laboratories should be observed.
- 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. DRG can, therefore, not be held responsible for any damage resulting from this.