DRG® Vitamin B6 (Pyridoxin) (BIO-4885)

USA: RUO

Revised 7 Dec. 2010 rm (Vers. 3.1)

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

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

INTENDED USE

The Vitamin B_6 Kit is a microtiter plate test kit based on a microbiological assay which measures the vitamin B_6 content (pyridoxine, pyridoxal, pyridoxamine) 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 vitamin B_6 content. For use in human and veterinary research.

PRINCIPLE OF THE TEST

The serum samples are enzymatically pre-treated in order to determine the total vitamin B_6 content. The diluted samples are transferred in the wells of a microtiter plate coated with Saccharomyces cerevisiae which metabolizes vitamin B_6. The addition of vitamin B_6 in either standards, controls or samples gives a vitamin B_6-dependent growth response until vitamin B_6 is consumed. After incubation at 30°C for 44 - 48 h, the growth of Saccharomyces cerevisiae is measured turbidimetrically at 610 - 630 nm (alternatively at 540 - 550 nm) in an ELISA-reader and a standard curve is generated from the dilution series.

The amount of vitamin B_6 is directly proportional to the turbidity.

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MATERIAL SUPPLIED

Label	Kit Components	Quantity
PLATE	One Saccharomyces cerevisiae precoated microtiter plate, ready to use	12 x 8 wells
SOL	Sample preparation buffer 5 ml, ready to use	4 x
ENZ	Enzyme, lyophilized	4 x
DIL	Water 30 ml	4 x
ASYMED	Vitamin B_6 assay medium	4 x
STD	Vitamin B_6 standard	4 x
CTRL1	Control 1	4 x
CTRL2	Control 2	4 x
FOL	Cover plastic foil	4 x
FRA	Replacement holder for 96-well plates	1 x

MATERIAL REQUIRED BUT NOT SUPPLIED

- Incubator with a dark incubation chamber, 30 °C
- Water bath or thermo block, 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.

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- Store unused strips in the original package with dry bag securely closed at 2-8 °C to prevent contamination or moisture exposure.
- 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], controls [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 [STD] as well as a sample and controls [CTRL1, CTRL2] 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 [PLATE] and materials that have been in contact with samples should be handled and disposed as potentially infectious.
- Signs for reagent damage: The highest standard should have an absorption higher than 0.6 Extinktion units (A_{630nm} > 0,6).

SAMPLE PREPARATION

Notes

- 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, 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 x g before assaying to obtain fat free serum as far as possible.
- Samples should be vortexed and then centrifuged (5 min at 10000 g) prior to measurement and the resulting supernatant used in the test.

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Sample preparation

- Resuspend the enzyme [ENZ] with sample preparation buffer [SOL]: add 4 ml sample preparation buffer [SOL] in the flask containing the lyophilized enzyme [ENZ], close and vortex.

- Add 300 µl serum or control [CTRL1, CTRL2] to 300 µl of the prepared enzyme solution, shake and incubate for 30 min at 37 °C in the dark. Afterwards, heat to 95°C for 30 min, cool quickly and centrifuge for 10 min at 10 000 x g.

- Take 100 µl from the supernatant of the treated serum sample, add 400 µl water [DIL] and mix.
- The sample treatment and dilution results in a final dilution of 1:10 (= 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. Return unused strips and any unused test kit component to the original foil bag, reseal them together with the desiccant provided, and put in the refrigerator.
- Bring all necessary reagents to room temperature.

Water [DIL] for medium [ASYMED], standard [STD] and controls [CTRL1, CTRL2]

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

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 an discard.
- Add 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 95 °C for 5 min, while shaking well at least twice. It is important to make sure that the medium bottle [ASYMED] is firmly closed at all times.
- Quickly cool the medium bottle [ASYMED] 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).

Control [CTRL1, CTRL2]

- The control must be freshly prepared before the test.
- Open the bottle of control [CTRL1, CTRL2], place the screw-top lid upside-down on the work bench.
- Add 0.75 ml water [DIL] from the test kit to the control bottle [CTRL1, CTRL2], close the bottle and dissolve by vortexing the bottle (= control).
- Treat the control afterwards as the sample is treated:

  Add 300 µl control to 300 µl of the prepared enzyme solution, shake and incubate for 30 min at 37 °C in the dark. Afterwards, heat to 95°C for 30 min, cool quickly and centrifuge for 10 min at 10 000 x g.

  Take 100 µl from the supernatant of the treated control, add 400 µl water [DIL] and mix.

- The control treatment and dilution results in a final dilution of 1:10 (= dilution factor).
- For the concentration of the controls [CTRL1, CTRL2] please see control specification.

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:

Vitamin B1	[µg/L]	Water [DIL]		Standard		Total volume
		[µl]	**+**	**[µl]**	**=**	**[µl]**
Blank:	0	940	+	0	=	940
Standard 1:	0.36	940	+	60	=	1000
Standard 2:	1.2	400	+	100	=	500
Standard 3:	1.8	350	+	150	=	500
Standard 4:	2.4	300	+	200	=	500
Standard 5:	3.6	200	+	300	=	500

Test Initiation

- Take as many microtiter strips as needed from the kit and put them in the second microtiter strip holder. Return unused strips to the original foil bag, reseal them together with the desiccant provided, and store at 2 – 8 °C to prevent contamination or moisture exposure.

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- A medium solution is sufficient for 6 strips.
- Put 150 µl Vitamin B_6 assay medium [ASYMED] in the cavities.
- Add 150 µl standard [STD], control [CTRL1, CTRL2], and sample, respectively, in the cavities. Pre-rinse the pipette tip with standard and sample solution respectively.
- Carefully seal the plate with plastic foil [FOL].
  Important: the cavities must be made airtight by pressing down with the hand!
- Keep at **30 °C for 44 - 48 hrs** in an incubator.

Measurement

- Securely press the foil down with the hand.
- Upturn the plate [PLATE] onto a tabletop and shake the germination well.
- Turn the plate [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 plate may be stored for a maximum of 48 hrs in the refrigerator before measuring the turbidity.

To prevent time-loss through holidays or weekends, the microtiter plate may also be evaluated after 60 hrs incubation.

REFERENCES / LITERATURE

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5. Selhub J et al. (1995) Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. N Engl J Med 332:286-291

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GENERAL NOTES ON THE TEST AND TEST PROCEDURE

This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.

Test components contain organic solvents. Contact with skin or mucous membranes must be avoided.

All reagents in the test package are for research use only.

Reagents should not be used after the date of expiry stated on the label.

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 variations of the test procedure that are not coordinated with the producer may influence the results of the test. DRG can, therefore, not be held reliable for any damage resulting from this.

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