DRG® Vitamin Niacin (BIO-4882)

CE

USA: RUO

Revised 28 Feb. 2011 rm (Vers. 4.1)

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 Niacin test is a microtiter plate test kit based on a microbiological assay which measures the total niacin content (nicotinic acid and nicotinamide) 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 niacin content.

PRINCIPLE OF THE TEST

Serum samples are diluted and added into the microtiter plate wells coated with Lactobacillus plantarum which metabolizes niacin. The presence of niacin both in standards and samples gives a niacin-dependent growth response. 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.

A dose response curve of absorbance unit (optical density, OD at 610 nm) vs. concentration is generated using the values obtained from standard.

DRG International Inc., USA

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MATERIAL SUPPLIED

Label	Kit Components	Quantity
PLATE	One Lactobacillus plantarum precoated microtiter plate, ready to use	12 x 8 wells
SOL	Sample stabilizing solution 5 mL, ready to use	4 x
DIL	Water 30 mL	4 x
ASYMED	Niacin assay medium	4 x
STD	Niacin standard	4 x
FOL	Cover plastic foil	4 x
FRA	Replacement holder for 96-well plates	1 x
CTRL1	Niacin Control 1	4 x
CTRL2	Niacin Control 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)

PREPARATION AND STORAGE OF REAGENTS

- Store test kit / reagents at 2 °C - 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, controls, water, sample stabilizing solution) in the test kit and store at 2 °C - 8 °C.
- Take as many microtiter strips as needed from kit. Store unused strips in the original package with dry bag securely closed at 2 °C - 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).
- Water quality is extremely important. Only the water delivered with the test kit should be used for medium [ASYMED], standard [STD] and control [CTRL1, CTRL2] reconstitution.
- It is essential to run a standard curve for each separate assay.
- It is recommended to run standard curve, controls as well as samples in duplicate.
- 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.
- By finishing the test, the used microtiter plates [PLATE] should be autoclaved.
- 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 °C - 8 °C until measurement. The samples are stable for 3 days at 2 °C - 8 °C in the dark. Niacin itself can be stored for longer at 2 °C - 8 °C, but not the serum. Therefore, samples should be frozen at -20°C for longer storage.
- 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 with visible amounts of precipitates should be centrifuged (5 min at 10000 g) prior to measurement and the resulting supernatant should be used in the test.

Sample dilution

Serum samples and controls [CTRL1, CTRL2] should be diluted 1 : 4 (= dilution factor) with sample stabilizing solution [SOL] from the kit prior to analysis:

100 µL sample + 300 µL sample stabilizing solution [SOL]

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], controls [CTRL1, CTRL2] and standard [STD]

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 and discard.
- Add 10 mL of water [DIL] from the test kit to the assay medium [Assay Medium], securely close the bottle and shake well. The amount is sufficient for 6 strips.
- Heat the bottle with medium [Assay Medium] 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 [Assay Medium] is firmly closed at all times.
- Quickly cool the medium bottle [Assay Medium] to under 30 °C.
- Filter the medium [Assay Medium] sterilely with a 0.2 µm filter in a 15 mL centrifuge test tube.

Standard [STD]

Before the test, freshly prepare the standard curve solutions:

- Open the bottle of standard [STD], remove seal. Dispose of screw-top lid and seal.
- Add x mL (x = see QS test kit data sheet) of water [DIL] from the test kit to the standard bottle [STD], close the bottle and dissolve by repeatedly (2-3 times= standard) vortexing it.
- 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:

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Niacin	[µg/L]	Water [DIL]		Standard [STD]		Total volume
		[µl]	**+**	**[µl]**	**=**	**[µl]**
Blank:	0	500	+	0	=	500
Standard 1:	4	450	+	50	=	500
Standard 2:	8	400	+	100	=	500
Standard 3:	16	300	+	200	=	500
Standard 4:	24	200	+	300	=	500
Standard 5:	40	0	+	500	=	500

Controls [CTRL1, CTRL2]

- The controls must be freshly prepared before use in the test.
- Open the bottle of controls [CTRL1, CTRL2], remove seal. Dispose of screw-top lid and seal.
- Add 1.25 mL of water [DIL] from the test kit to the control bottle [CTRL1, CTRL2], close the bottle and dissolve by vortexing the bottle (= control 1, control 2).
- Treat the controls [CTRL1, CTRL2] afterwards as the samples are treated.

Test Initiation

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

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Measurement

- Securely press the foil [FOL] down with the hand.
- Upturn the plate [PLATE] onto a tabletop and shake the germination well.
- Turn the plate over again and carefully remove the foil [FOL], beginning with the upper right corner and pulling diagonally backwards at an angle of 180°.
- 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

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

Niacin in µg/L = Value from the standard curve x sample dilution factor

Reference value for human serum

Serum (n = 83): Niacin (total soluble forms): 17 - 85 µg/L (Median ± 2 SD)

Please note:

A concentration range of 16 - 160 µg/L Niacin is covered at a sample dilution 1:4.

We recommend each laboratory to develop its own normal range. The values mentioned above are only for orientation and can deviate from other published data.

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Revised 28 Feb. 2011 rm (Vers. 4.1)

REFERENCES / LITERATURE

1. Morris M C et al. (2004) Dietary niacin and the risk of incident Alzheimer´s disease and of cognitive decline. J Neurol Neurosurg Psychiatry 75: 1093-1099

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 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.

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.

Version 2011-01-31~rm

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