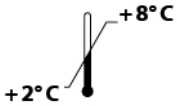
Manual

ID-Vit®*Niacin Assay Kit*

Microbiological test kit for the determination of total niacin (nicotinic acid / nicotinamid acid) in serum using a Lactobacillus coated microtitre plate

Valid from 03.07.2012

REF **KIF003**



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®*Niacin* 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. **For use in human and veterinary medicine and in research. The Niacin Assay kit is For Research Use Only.**

2. INTRODUCTION

Niacin (nicotinic acid and nicotinamide) is used by the body to form coenzymes such as nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phospate ($NADP^+$). As many as 200 enzymes require the two coenzymes, NAD^+ and $NADP^+$, mainly to accept or donate electrons for redox reactions. NAD^+ functions most often in reactions involving the degradation (catabolism) of carbohydrates, fats, proteins, and alcohol to produce energy. $NADP^+$ functions more often in biosynthetic (anabolic) reactions, such as in the synthesis of fatty acids and cholesterol. Since almost every metabolic pathway uses either NAD^+ or $NADP^+$, it is not surprising to find signs and symptoms of niacin deficiency in severe metabolic disorders. The worst of these is pellagra which is characterized by the four D's, representing: Dermatitis, Diarrhoea, Dementia and Death.

Niacin deficiency syndromes

Symptoms of **minor niacin deficiency**:

- Loss of appetite
- Depression
- Dementia
- Insomnia
- Weakness
- Irritability

Severe niacin deficiency may cause pellagra. The term pellagra is derived from the Italian words "pelle agra" meaning "rough" or "smarting skin". Pellagra is characterized by symptoms such as:

- Glossitis
- Sore, swollen, purple-red tongue
- Skin lesions primarily located on sun-exposed areas

Niacin as cholesterol lowering drug

Niacin increases HDL cholesterol and reduces LDL cholesterol and triglycerides. When taken in conjunction with another cholesterol medication, diet or exercise, niacin has been proven to reduce „bad" cholesterol levels. A niacin-statin combination therapy substantially improves 4 major lipoprotein levels associated with atherosclerotic disease (Insull et al. 2004). The drug combination had good records in clinical trials for reduction in cardiovascular events and improvement in progression/regression of coronary lesions.

Indications

- Deeply pigmented skin on sun-exposed areas
- Alcohol abuse
- Dementia
- Dry skin and mouth
- Numbness of the extremities
- Inflammation of the mucous membranes of the tongue and mouth
- Digestive disorders

Niacin can be synthesized in the body from tryptophan, whereby the conversion requires the presence of thiamine, pyridoxine, and riboflavin. Any deficiency in these vitamins can affect the niacin metabolism.

3. 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 [STD] 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. Niacin present in the patient samples is determined directly from this curve.

4. MATERIAL SUPPLIED

Catalog No	Label	Kit Components	Quantity
KIF003MTP	PLATE	One ***Lactobacillusplantarum***-pre-coated microtiter plate, ready to use	12 x 8 wells
KIF003SO	SOL	Sample stabilizing solution 5 ml, ready to use	4 x
KIF003DI	DIL	Water 30 ml	4 x
KIF003ME	ASYMED	Niacin-Assay-Medium	4 x
KIF003ST	STD	Niacin -Standard	4 x
KIF003FO	FOL	Cover plastic foil	4 x
KIF003FR	FRA	Replacement holder for 96-well plates	1 x
KIF003KO1	CTRL1	Niacin Control 1	4 x
KIF003KO2	CTRL2	Niacin Control 2	4 x

5. 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 polyethersulfon filter with a sterile tip
- 15 ml centrifugal tubes, sterile (e.g. Falcon tubes)

6. 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 reagent (standard, medium, controls, water, sample stabilizing solution) in the test kit and store at 2-8°C.

Take as many microtiter strips as needed from kit. Store unused strips in the original package bag 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.

7. 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.
- For sterile filtration, only a sterile polyethersulfone filter must be used.
- 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 (A630nm > 0,6)

8. SAMPLE PREPARATION

Notes

Patient serum is used for analysis.
Original samples should be kept light-protected at 2-8°C until measurement. The samples are stable for 3 days at 2-8°C in the dark. Niacin itself can be stored for longer at 2 - 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.

8.1 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]

9. 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.

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], 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 the test.
- Take the dry bag out of medium vial [ASYMED] by tweezers, shake off an discard.
- Add 10 ml of water [DIL] from the test kit to the assay medium, securely close the bottle and shake well. The amount is sufficient for 6 strips.
- Heat the bottle with 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 is firmly closed at all times.
- Quickly cool the medium bottle to under 30 °C.
- Filter the medium with a 0.2 µm sterile filter in a 15 ml centrifugal 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] from the test kit into 6 sterile reaction vials (capacity 1.5 – 2.0 ml) and then pipet the standard to the vials. Prepare a standard curve using the following scheme:

Niacin [µg/L]		Water [DIL] [µl]	+	Standard [STD] [µl]	=	Total volume [µl]
Blank:	**0**	500	+	0	=	500
Standard 1:	**2**	475	+	25	=	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.

- Pipette 150 µl of the 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.

9.2. 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 in 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** for 48 hrs in an incubator.

9.3. 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 lower, left 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.

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

Niacin in µg / l = Value from the standard curve x 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 8 - 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.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n = 6)		
	Niacin [µg/l]	VK [%]
Sample 1	64	2.9
Inter-Assay (n = 5)		
	Niacin [µg/l]	VK [%]
Sample 1	64	3.4

Recovery

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

n = 9

Sample	Mean value measured in original sample [µg/L]	Spike [µg/L]	Niacin expected [µg/L]	Niacin detected [µg/L]	Recovery Rate [%]
A	21.5	60	81.5	77	93
		120	141.5	134	94
		180	201.5	203	101
				Recovery rate in total [%]	**96**

n = 9

Sample	Mean value measured in original sample [µg/L]	Spike [µg/L]	Niacin expected [µg/L]	Niacin detected [µg/L]	Recovery Rate [%]
B	16.9	60	76.9	76	99
		120	136.9	136	100
		180	196.9	199	101
				Recovery rate in total [%]	**100**

n = 9

Sample	Mean value measured in original sample [µg/L]	Spike [µg/L]	Niacin expected [µg/L]	Niacin detected [µg/L]	Recovery Rate [%]
C	19.9	60	79.9	79	99
		120	139.9	145	104
		180	199.9	203	102
				Recovery rate in total [%]	**102**

n = 10

Sample	Mean value measured in original sample [µg/l]	Spike [µg/L]	Niacin expected [µg/L]	Niacin detected [µg/L]	Recovery Rate [%]
D	18.3	60	78.3	82	106
		120	138.3	139	100
		180	198.3	195	98
				Recovery rate in total [%]	**102**

Linearity

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

n = 2

Sample	Dilution	Niacin expected [µg/L]	Niacin detected [µg/L]
A	4	128	128
	8		138
	16		137
D	8	196	196
	16		193
	24		203
	32		188

12. REFERENCES

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

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

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

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.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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