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Manual

ID-Vit® Niacin

Microbiological test kit for the determination of total free niacin (nicotinic acid/nicotinamid acid) in serum using a Lactobacillus plantarum-coated microtitre plate
For use in human and veterinary medicine and in research

Valid from 2019-05-23

KIF003

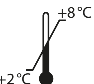

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Table of Contents

1. INTENDED USE 17

2. INTRODUCTION 17

3. PRINCIPLE OF THE TEST 18

4. MATERIAL SUPPLIED 18

5. MATERIAL REQUIRED BUT NOT SUPPLIED 19

6. PRECAUTIONS 19

7. STORAGE AND PREPARATION OF REAGENTS 20

7.1 Water 20
7.2 Preparation of the controls 20
7.3 Preparation of the standard curve 20
7.4 Preparation of the sterile assay medium 21
7.5 Microtiter plate [PLATE] 21

8. SAMPLE STORAGE AND PREPARATION 22

8.1 Sample dilution 22

9. ASSAY PROCEDURE 22

9.1 Test preparations 22
9.2 Test procedure 22
9.3 Measurement 23

10. EVALUATION OF RESULTS 23

10.1 Calculation 23
10.2 Quality control 24

11. LIMITATIONS 24

12. PERFORMANCE CHARACTERISTICS 24

12.1 Precision and reproducibility 24
12.2 Recovery 24
12.3 Linearity 26

13. REFERENCES 26

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE 27

1. INTENDED USE

ID-Vit® Niacin is a microtiter plate test kit based on a microbiological method which measures the total free niacin content in serum. The test kit contains the standard and all reagents required to perform the test. It is sufficient for 96 determinations including standard curves. An ELISA reader is required for the evaluation of the results. For use in human and veterinary medicine and in research. For *in vitro* diagnostic 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 Ds, representing dermatitis, diarrhoea, dementia and death.

Niacin deficiency syndromes

Symptoms of minor niacin deficiency:

- Loss of appetite
- Depressiveness
- 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, ni-

acin 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

The serum samples are diluted and then transferred into the wells of a microtiter plate coated with *Lactobacillus plantarum*. The addition of niacin in either standards or samples gives a niacin-dependent growth response until niacin is consumed. After incubation at **37 °C** for **48 h**, the growth of *Lactobacillus plantarum* is measured turbidimetrically at 610–630 nm (alternatively at 540–550 nm) in an ELISA reader and compared to a standard curve generated from the dilution series. The amount of niacin is directly proportional to the turbidity.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KIF003	PLATE	*Lactobacillus plantarum*-precoated microtiter plate	1 x
KIF003	SOL	Sample stabilising solution	4 x 5 ml
KIF003	DIL	Water	4 x 30 ml
KIF003	ASYMED	Niacin assay medium	4 x
KIF003	STD	Niacin standard, lyoph.	4 x
KIF003	FOL	Adhesive cover foil	4 x

Cat. No.	Label	Kit components	Quantity
KIF003	FRA	Replacement holder for microtiter strips	1 x
KIF003	CTRL1	Niacin control 1, lyoph.	4 x
KIF003	CTRL2	Niacin control 2, lyoph.	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)
- Calibrated precision pipettors and sterile 20–1000 µl tips
- 5 ml and 10 ml pipets
- 1.5–2 ml reaction vials, sterile
- 0.2 µm sterile polyethersulfone (PES) filter with a sterile disposable syringe
- 15 ml centrifuge tubes, sterile (e. g. Falcon tubes)
- Biocentrifuge (10 000 *g*)

6. PRECAUTIONS

- As the test is based on a microbiological method, the general guidelines for sterile work should be observed as far as possible (preferably work in a sterile bench / PCR hood, use of sterile instruments or equipment).
- GLP (Good Laboratory Practice) guidelines have to be observed.
- Water quality is extremely important for the test. Only the water delivered with the test kit [DIL] should be used.
- For sterile filtration, only a sterile polyethersulfone filter must be used.
- It is essential to run a standard curve for each separate assay.
- Controls should be measured with each assay.
- We recommend measurements in duplicate.
- If a higher dilution results in a higher value measured, inhibitors like antibiotics might be present.
- Reagents should not be used beyond the expiration date shown on the label.
- Wear gloves during the test.

- Used microtiter stripes [PLATE] and materials that have been in contact with patient samples should be handled and disposed as potentially infectious.

7. STORAGE AND PREPARATION OF REAGENTS

- Store test kit and reagents at 2–8 °C.
- Prepare reagents freshly and use them immediately after preparation. Discard remaining unused reagents and waste in accordance with country, federal, state, and local regulations.
- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. Prepare only the appropriate amount necessary for each run. The kit can be used up to 4 times within the expiry date stated on the label.

7.1 Water

- Water [DIL] (for medium [ASYMED], standard [STD] and controls [CTRL1, CTRL2])
- Push the lid up and pull it back to the rim of the glass, then twist the whole cap off.

7.2 Preparation of the controls

- The lyophilised controls [CTRL1, CTRL2] have to be resuspended with each 1,25 ml water [DIL] from the test kit, then homogenise using a vortex.
- After reconstitution, the controls are treated like samples.
- The concentration of the controls changes from lot to lot and is stated in the product specification.

7.3 Preparation of the standard curve

- For the preparation of the standard curve, standard concentrate is needed. To prepare standard concentrate, resuspend the lyophilised standard [STD] with x ml (x = please see the enclosed quality control protocol for the volume needed) water [DIL] supplied with the test kit, then homogenise using a vortex.
- Prepare a standard curve in 6 sterile reaction tubes (1.5–2 ml volume) from standard concentrate and water [DIL] following the scheme depicted in the table below:

Niacin [µg/l]		Water [DIL] [µl]	+	Standard concentrate [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

7.4 Preparation of the sterile assay medium

- Fresh sterile assay medium has to be prepared each time before performing a test.
- Remove lyophilised assay medium from the desiccant bag in the assay medium bottle by taking the bag with a forceps and shaking it whilst still inside the bottle. Then remove the clean desiccant bag and discard it.
- Add 10 ml water [DIL] to the assay medium bottle [ASYMED], close the bottle firmly and shake it. This amount is sufficient for 6 microtiter stripes.
- Heat the medium bottle in a water bath at 90–100 °C for 5 min, shake well at least 2 times during this incubation time. Take care that the medium bottle is always firmly closed.
- Quickly cool the medium bottle to < 30 °C (at 2–8 °C for 10 min).
- Filter the medium using a disposable syringe (10 ml) and the 0.2 µm PES filter into a sterile centrifuge tube (15 ml, e. g. Falcon).
- After this preparation, the sterile assay medium can be used in the test.

7.5 Microtiter plate [PLATE]

- Store the microtiter plate [PLATE] in the aluminium packaging containing the desiccant bag at 2–8 °C.
- The microtiter plate [PLATE] has to be protected from humidity and contamination.
- Take care that the aluminium packaging is not damaged.
- Carefully close the aluminium packaging after opening.

- Take only the microtiter stripes needed directly before usage to avoid contamination

8. SAMPLE STORAGE AND PREPARATION

- Use serum for analysis.
- Samples are stable at 2–8 °C for three days 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 *g* before assaying to obtain fat free serum as far as possible.
- Samples should be centrifuged (at least 5 min at 10 000 *g*) prior to measurement. Use the resulting supernatant in the test.

8.1 Sample dilution

Take 100 µl from the sample/control, add 300 µl sample stabilising solution [SOL] and mix. This results in a total dilution of 1:4 (= sample dilution factor).

9. ASSAY PROCEDURE

9.1 Test preparations

Take as many microtiter strips as needed from kit. Return unused strips and any unused test kit component to the original packaging, and put in the refrigerator. Bring all necessary reagents to room temperature.

9.2 Test procedure

- Take as many microtiter strips as needed from the kit and put them in the second microtiter strip holder [FRA].
- Put 150 µl sterile assay medium into the cavities.
- Add each 150 µl of the prepared standard curve, samples and controls into the respective cavities.Pre-rinse each pipet tip with standard, control or sample solution, respectively.
- Carefully seal the plate with adhesive cover foil [FOL]. Important: the cavities must be made airtight by pressing the foil down with the hand!
- Keep at 37 °C for 48 h in an incubator.

9.3 Measurement

- Press the adhesive cover foil [FOL] firmly down again with the hand.
- Upturn the microtiter plate [PLATE], put it onto a tabletop and shake the microbes well.
- Turn the microtiter plate [PLATE] over again and carefully remove the adhesive cover foil [FOL]. 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 pipet tip or a needle.
- Read turbidity in an ELISA reader at E 610–630 nm (alternatively at E 540–550 nm).

Please note

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

10. EVALUATION OF RESULTS

We recommend to use the 4 parameter algorithm to calculate the results. The sample dilution factor has to be considered for data evaluation.

The blank should have an optical density < standard 1. It serves as optical control to exclude contaminations and is not included in the calculation of results.

10.1 Calculation

Niacin in µg/l = value from the standard curve × sample dilution factor (4)

Reference value for human serum

Based on studies of serum samples of apparently healthy persons (n = 83), the following values were estimated.

Niacin: 17–85 µg/l (Mean ± 2 SD)

Please note

A concentration range of 8–160 µg/l niacin is covered at a sample dilution of 1:4.

We recommend each laboratory to develop its own normal range as normal ranges strongly depend on the choice of the patient collective. The values mentioned above are only for orientation and can deviate from other published data.

10.2 Quality control

The extinction of the highest standard has to be > 0.6.

Results, generated from the analysis of control samples, should be evaluated for acceptability. The results for the samples may not be valid if within the same assay one or more values of the quality control sample or the highest standard are outside the acceptable limits.

11. LIMITATIONS

Whole blood cannot be used in the assay.

12. PERFORMANCE CHARACTERISTICS

The following performance characteristics have been collected using human serum samples.

12.1 Precision and reproducibility

Intraassay (n = 6)

	Niacin [µg/l]	CV [%]
Sample	64	2.9

Interassay (n = 5)

	Niacin [µg/l]	CV [%]
Sample	64	3.4

12.2 Recovery

Samples from 4 patients were differently diluted (20, 40, 80, 120, 160), spiked with niacin and analysed. The mean values are shown below.

Sample (n=9)	Mean value original sample [µg/l]	Spike [µg/l]	Niacin expected [µg/l]	Niacin measured [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**

Sample (n=9)	Mean value original sample [µg/l]	Spike [µg/l]	Niacin expected [µg/l]	Niacin measured [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**

Sample (n=9)	Mean value original sample [µg/l]	Spike [µg/l]	Niacin expected [µg/l]	Niacin measured [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**

Sample (n=9)	Mean value original sample [µg/l]	Spike [µg/l]	Niacin expected [µg/l]	Niacin measured [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**

12.3 Linearity

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

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

13. REFERENCES

1. Strohecker, R. & Henning, H., **1963**. Vitamin-Bestimmungen. Erprobte Methoden. Weinheim/Bergstraße: *Verlag Chemie GmbH*.
2. Singer, D. et al., 2012. Defective intestinal amino acid absorption in Ace2 null mice. American journal of physiology. *Gastrointestinal and liver physiology*, **303**(6), pp.G686-95.
3. Morris, M.C. et al., 2004. Dietary niacin and the risk of incident Alzheimer's disease and of cognitive decline. *Journal of neurology, neurosurgery, and psychiatry*, **75**(8), pp.1093–9.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- *ID-Vit*® is a trademark of Immundiagnostik AG.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Do not interchange different lot numbers of any kit component within the same assay.
- The guidelines for medical laboratories should be followed.
- 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. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.
- Control samples should be analysed with each run.
- The assay should always be performed according to the enclosed manual.

Used symbols:

Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with

Manufacturer

Contains sufficient for <n> tests

Lot number

Use by

Attention

Consult instructions for use

Consult specification data sheet