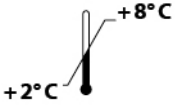
Manual

ID-Vit®
Pantothenic Acid Assay Kit

Microbiological test kit for the determination of total pantothenic acid in serum using a Lactobacillus plantarum coated microtitre plate

Valid from 13.03.2012

REF **KIF004**



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1. INTENDED USE

ID-Vit®*Pantothenic acid* is a microtiter plate test kit based on a microbiological assay which measures the total pantothenic 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 pantothenic acid content. **For use in human and veterinary medicine and in research. The Pantothenic Acid Assay kit is For Research Use Only.**

2. INTRODUCTION

Pantothenic acid is the reactive thiol function of CoA and ACP

Pantothenic acid (vitamin B_5) is synthesized by most microorganisms and plants from pantoic acid. The vitamin is an integral part of 4'-phosphopantheine, which is a component of **coenzyme A** (CoA). CoA plays a key role in the metabolism of numerous compounds, especially lipids and the ultimate catabolic disposition of carbohydrates and ketogenic amino acids. About 80% of the vitamin in animal tissues is in CoA form, and the rest exists mainly as phosphopanthetheine and phosphopantethenate.

Another essential role of pantothenic acid is its participation in the 4'-phosphopantheine moiety of acyl carrier protein (ACP), where the phosphodiester-linked prosthetic group uses the sulfhydryl terminus to exchange with malonyl-CoA to form an ACP-S malonyl thioester, which can chain elongate during fatty acid biosynthesis.

Pantothenic acid deficiency

Pantothenic acid deficiency is exceedingly rare. Because of its rarity, most information about pantothenic acid deficiency has been obtained from experiments: Pantothenic acid deficiency has been induced in humans by use of a metabolic antagonist, w-methyl pantothenic acid along with a pantothenic acid-deficient diet. Subjects became irascible and developed postural hypotension and rapid heart rate on exertion, epigastric distress with anorexia and constipation, numbness and tingling of the hands and feet. Because pantothenic acid is involved with so many vital processes in the body, it is not surprising that a broad number of complications might result from deficiency.

From recent research it is known that the pantothenic acid derivative, pantethine (two molecules of pantetheine joined by a disulfide bond), has a hypocholesterolemic effect. A metabolic antagonist of pantothenic acid, pantoyl γ-amino butyric acid (called pantoyl-GABA), is widely used in Japan as an anti-dementia drug for treating cognitive impairments in pathological states such as Alzheimer's disease, presumably through increasing cholinergic activity in vivo.

Indications: Suspicion of inadequate intake of pantothenic acid (e.g. in patients at high risk for malnutrition)

3. PRINCIPLE OF THE TEST

Serum samples are diluted and added into the microtiter plate wells coated with Lactobacillus plantarum which metabolizes pantothenic acid. The presence of pantothenic acid both in standards [STD] and samples gives a pantothenic acid-dependent growth response. After incubation at 37°C for 24 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. Pantothenic acid present in the patient samples is determined directly from this curve.

4. MATERIAL SUPPLIED

Catalog No	Label	Kit Components	Quantity
KIF004MTP	PLATE	One ***Lactobacillus plantarum***-precoated microtiter plate, ready to use	12 x 8 wells
KIF004SO	SOL	Sample stabilizing solution 5 ml, ready to use	4 x
KIF004DI	DIL	Water 30 ml	4 x
KIF004ME	ASYMED	Pantothenic acid assay medium	4 x
KIF004ST	STD	Pantothenic acid - standard	4 x
KIF004FO	FOL	Cover plastic foil	4 x
KIF004FR	FRA	Replacement holder for 96-well plates	1 x
KIF004KO1	CTRL1	Control 1 Pantothenic acid	4 x
KIF004KO2	CTRL2	Control 2 Pantothenic acid	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 reagents (standard, medium) 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 as well as for sample preparation.
- 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 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.
- 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. Pantothenic acid 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 at10000 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 : 8 (= dilution factor) with sample stabilizing solution [SOL] from the kit prior to analysis:

50 µl sample or control [CTRL1, CTRL2] + 350 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], 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 the test.
- Take the dry bag out of medium vial [ASYMED] by tweezers, shake off an discard.
- Add 10 ml of water 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 95 °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 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], place the screw-top lid upside-down on the work bench.
- Add x ml (x = see QStest kit data sheet) of water [DIL] from the test kit to the standard bottle [STD], 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:

Pantothenic acid [µg / l]		Water [DIL] [µl]	+	Standard [STD] [µl]	=	Total volume [µl]
Blank:	**0**	975	+	0	=	975
Standard 1:	**2,3**	975	+	25	=	1000
Standard 2:	**4.6**	950	+	50	=	1000
Standard 3:	**18.4**	400	+	100	=	500
Standard 4:	**27.6**	350	+	150	=	500
Standard 5:	**36.8**	300	+	200	=	500

Controls [CTRL1, CTRL2]

- The control must be freshly prepared before 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 control afterwards as the sample is 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 pantothenic acid assay medium [ASYMED] in the cavities.
- Add 150 µl of standard [STD], control [CTRL1, CTRL2] respectively, sample in the 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 24 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 24 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.

Pantothenic acid in µg/l = Value from the standard curve x dilution factor

11. EXPECTED VALUES

Range of concentration

The concentration of pantothenic acid was determined in 74 samples of different blood donors. The median value was 91.4 (81.4)µg/L. The 2-SD area was 36 to 147 µg/L. Figure 1 shows the distribution of the values.

Number of samples	74
Mean	91.4
Median	81.35
SD	27.7
MW-2* SD	36.0
MW+2*SD	146.8

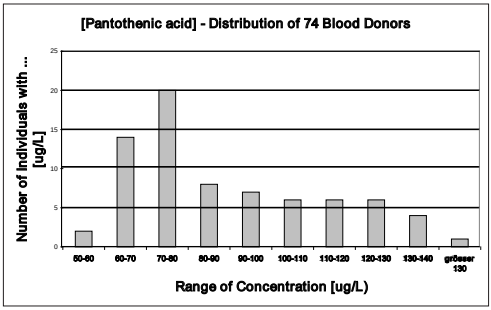

Fig. 1: Distribution of pantothenic acid values in blood donor samples

Please note: A concentration range of 18.4 - 294.4 µg/L pantothenic acid is covered at a sample dilution 1 : 8. 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.

12. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n = 28)		
	Pantothenic acid [µg/l] Mean value	VC [%]
Sample 1	81.0	3.0
Inter-Assay (n = 5)		
	Pantothenic acid [µg/l] Mean value	VC [%]
Sample 1	92.36	4.91

Recovery

Samples from 3 patients were spiked with Pantothenic acid and analyzed. The mean values are shown below:

n = 5

Sample	Mean value measured in original sample [µg/L]	Spike [µg/L]	Pantothenic acid expected [µg/L]	Pantothenic acid measured [µg/L]	Recovery Rate [%]
A	112.5	18.4	130.9	131.88	105
		36.8	149.3	141.47	79
		55.2	167.7	158.12	83
				Recovery rate in total [%]	**89**

n = 5

Sample	Mean value measured in original sample [µg/L]	Spike [µg/L]	Pantothensäure expected [µg/L]	Pantothensäure measured [µg/L]	Recovery Rate [%]
B	96.61	18.4	115.01	113.79	93
		36.8	133.41	133.80	101
		55.2	151.81	165.33	125
				Recovery rate in total [%]	**106**

n = 5

Sample	Mean value measured in original sample [µg/L]	Spike [µg/L]	Pantothensäure expected [µg/L]	Pantothensäure measured [µg/L]	Recovery Rate [%]
C	106.21	18.4	124.61	122.45	88
		36.8	143.01	138.62	88
		55.2	161.41	176.12	127
				Recovery rate in total [%]	**101**

13. REFERENCES

Burtis C A, Ashwood E R (Eds): Tietz Textbook of Clinical Chemistry, 3rd Edition, 1999

Coronel F et al. (1991) Treatment of hyperlipemia in diabetic patients on dialysis with a physiological substance. Am J Nephrol 11(1):32-6

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Assay 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.

Warranty Information

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