CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

100904

The AOAC Research Institute hereby certifies the performance of the test kit known as:

VitaFast® Pantothenic Acid Microbiological Microtiter Plate Test for the Determination of Pantothenic Acid

manufactured by
Institut fur Produktqualitaet GmbH
Wagner-Régeny-Str. 8
12489 Berlin
Germany

distributed by
R-Biopharm AG
An der neuen Bergstraβe 17
64297 Darmstadt
Germany

This method has been evaluated in the AOAC® *Performance Tested Methods*SM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above-mentioned method for a period of one calendar year from the date of this certificate (January 07, 2021 – December 31, 2021). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

January 07, 2021
Date

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KIT NAME(S)
VitaFast® Pantothenic Acid Microbiological Microtiter Plate Test for the Determination of Pantothenic Acid

CATALOG NUMBERS
P1005

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APPLICABILITY OF METHOD
Target analyte – B vitamin Pantothenic acid

Matrixes – (1 g) - Cereals, processed meats, multivitamin pills, powders, beverages like fruit juice & milk

Performance claims - The performance characteristics of VitaFast® Pantothenic Acid kit meet the following specifications:
1)Time required for completion of the sample extraction was 2 hours and less than 48 hours for the test implementation.
2) The test kit components are stable as indicated on the test kit labels (shelf life is 12 months).
3) Analytical Sensitivity was found at LOD 0.0035mg / 100 g as measured by 10 blank samples from 10 different lots. LOQ was set at 0.04 mg Ca-Pantothentate / 100 g sample, which corresponds to standard 1 of the curve.
4) Accuracy was investigated by analysis of reference materials from proficiency programs, internal reference materials, and also by commercial product analysis and spike recovery studies. In general recovery was within acceptable limits.
5) The VitaFast test kit was shown to have a high degree of precision, with inter-assay variances below 10 % for all matrixes.
6) The VitaFast plate test is not sensitive to temperature changes between 36 °C and 38 °C, incubation time between 24 and 28 hours, or assay medium volumes between 145 and 155 µl.

ORIGINAL CERTIFICATION DATE
October 28, 2009

CERTIFICATION RENEWAL RECORD
Renewed Annually through December 2021

METHOD MODIFICATION RECORD
1. **March 2017 Level 2**

SUMMARY OF MODIFICATION
1. **Location change to Wagner-Régeny-Str., Berlin**

Under this AOAC® *Performance Tested*℠ License Number, 100904 this method is distributed by:
R-Biopharm AG

Under this AOAC® *Performance Tested*℠ License Number, 100904 this method is distributed as:
VitaFast® Pantothenic Acid Microbiological Microtiter Plate Test for the Determination of Pantothenic Acid

PRINCIPLE OF THE METHOD (1)
Pantothenic acid is extracted from the sample and the extract is diluted. The diluted extract and the pantothenic acid assay - medium are pipetted into the wells of a microtiter plate which are coated with *Lactobacillus plantarum*. The growth of *L. plantarum* is dependent on the supply of pantothenic acid. Following the addition of pantothenic acid as a standard or as a compound of the sample, the bacteria grow until the vitamin is consumed. The incubation is done in the dark at 37 °C (98.6 °F) for 20 - 28 h. The intensity of metabolism or growth in relation to the extracted pantothenic acid is measured as turbidity and compared to a standard curve. The measurement is done using a microtiter plate reader at 610 - 630 nm (alternatively at 540 - 550 nm).

DISCUSSION OF THE VALIDATION STUDY (1)

The VitaFast® Pantothenic Acid test kit is calibrated according to a standard curve of five standard concentrations, using 4-parameter fitting software. The curve shown in figure 1 is typical. Variation within the curve is consistently minor, at a level of variance below 10 %. Stability is also demonstrated over the entire shelf life of the product, and regular quality tests ensure this is true for all lots produced.

Lot-to-lot tests show a high degree of repeatability across lots, throughout the entire shelf life. Not only was the result for the AACC reference material consistent across the four lots with a CV of less than 2 %, but CVs for the raw absorbance data of the standards across the four lots indicate little variation in the calibration of individual test. This demonstrates the excellent uniformity of the kits. Accuracy was established using recognized and reliable reference materials, as well as spike recovery data and analysis of various food products available on the market.

It was shown that small variations in test implementation did not significantly affect the performance of the test kit. The assay was sufficiently rugged across varying incubation times and temperatures, and reagent volumes which may be introduced non-purposefully by the operating technician. These ruggedness studies show that the test kit will still reliably produce high quality results under minor fluctuations in conditions. The test kit components showed excellent stability over a period of 12 months without any loss of analytical capacity.

Furthermore the test was not influenced by small changes above and below the environmental and operating parameters specified in the leaflet such as temperature, incubation time and volume.

In the independent laboratory study, the accuracy and repeatability of the VitaFast method is well-proven in the analysis of the reference material from NIST. There is however, a discrepancy between the result obtained by VitaFast® and the value which was declared on the package label, whereas method 945.74 produced a result that agreed with the label claim. Results of the AOAC extract on the VitaFast® plate were also lower than the label claim of the sample. This indicates the presence of an inhibitory substance in the sample. When inhibition is suspected, a spike recovery study may be carried out to confirm whether this is the case. Results of the internal validations show excellent recovery from several cereal matrixes, indicating that the inhibition observed in the independent study is a rare occurrence and not typical of the cereal matrix.

Table 6 Intra-assay variance of food samples (1)

Sample description	Concentration indicated on label (mg / 100g)	Mean result of dilutions in mg / 100 mg (n=3)	Coefficient of variation in %
Infant formula	1.76	2.1	4.1
Multi vitamin sweet (bonbon)	26.6	33.6	2.0
Cereals	5.55	5.5	1.5
Multi vitamin pills	400	465	1.4
Ham sausage 1	6.95	7.82	1.5
Ham sausage 2	6.95	6.89	2.2
Dextrose powder RM – Vit001 intern	6	9.2	4.6
Milk powder RM – Vit002 intern	3.8	4.4	5.4
Fruit juice	0.9	1.07	2.5
Energy drink	2	1.83	1.0
Multivitamin juice drink	3	3.48	0.8

Table 13: Data summary for pantothenic acid test in infant formula and cereal using AOAC official method 945.74, and VitaFast P1005. (1)

Sample (1)	Infant Formula (µg/g) (2)		Cereal (µg/g) (3)		Cereal (µg/g)[1] (4)	
	AOAC	VitaFast	AOAC	VitaFast	AOAC Extraction	VitaFast Extraction
1	26.02	47.30	32.88	11.70	10.90	9.90
2	30.86	65.20	37.84	12.80	12.25	8.85
3	47.08	40.50	31.75	13.40	11.65	9.75
4	56.37	79.20	30.64	11.40	11.60	8.10
5	50.72	41.25	40.50	13.90	15.90	9.65
6	37.53	37.35	27.63	13.40	14.50	9.40
7	72.87	56.30	40.56	14.70	15.45	9.80
8	50.51	48.30	27.47	13.60	13.05	9.10
Mean	46.50	51.93	33.66	13.11	13.16	9.32
Standard Deviation	14.98	14.30	5.34	1.11	1.898	0.612
95% Confidence (±)	10.38	9.91	3.70	0.77	1.315	0.424
Label Value[2]	48.7±7.3		35 µg/g			
RDA[3]	7 mg					

[1]A test added to test the AOAC extraction by VitaFast kit.
[2]Direct from label (infant formula) or calculated from label on box and its RDA (cereal).
[3]RDA, recommended daily allowance.

REFERENCES CITED

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