CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

101001

The AOAC Research Institute hereby certifies the method known as:

VitaFast® Biotin (B7) Microbiological Microtiter Plate Test for the Determination of Biotin

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 Research Institute *Performance Tested Methods*[SM] Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date December 2, 2022
Expiration Date December 31, 2023

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METHOD NAME
VitaFast® Biotin (B7) Microbiological Microtiter Plate Test for the Determination of Biotin

CATALOG NUMBER
P1003

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[3] Modification March 2017 (11)
Modification July 2022 reviewed internally by AOAC Research Institute.

APPLICABILITY OF METHOD
Target analyte – Biotin

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

Performance claims - The performance characteristics of VitaFast® Vitamin B7 (Biotin) 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 0.013 µg / 100 g as measured by 190 blank samples from 10 different lots. LOQ was set at 0.08 µg Biotin / 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 44 and 52 hours, or assay medium volumes between 145 and 155 µl.

ORIGINAL CERTIFICATION DATE
October 28, 2009

CERTIFICATION RENEWAL RECORD
Renewed Annually through December 2023.

METHOD MODIFICATION RECORD
1. **March 2017 Level 2**
2. **July 2022 Level 2**

SUMMARY OF MODIFICATION
1. **Location change to Wagner-Régeny-Str., Berlin.**
2. **Corrections made to Instructions for Use.**

Under this AOAC *Performance Tested Methods℠* License Number, 101001 this method is distributed by:
R-Biopharm AG

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VitaFast® Biotin (B7) Microbiological Microtiter Plate Test for the Determination of Biotin

PRINCIPLE OF THE METHOD (1)
Biotin is extracted from the sample and the extract is diluted. The diluted extract and the biotin 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 biotin. Following the addition of biotin 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 44 - 48 h. The intensity of metabolism or growth in relation to the extracted biotin 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® Vitamin B7 (Biotin) 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.

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 incubation time and volume.

In the independent laboratory study, accuracy and repeatability of the VitaFast method was well-proven in the analysis of the NIST material. Although the results of the reference method were slightly higher than that of the VitaFast method, both were within the acceptable limits of the material. However, when the FDA extract was analyzed on the VitaFast plate, the results were lower than expected. The source of this discrepancy is unknown. Nevertheless, when implemented as outlined in the product insert, the VitaFast method performed as expected for the NIST sample.

The VitaFast method produced a very low result for the AACC material when the enzymatic extraction was followed, while the reference method produced a result within the specifications of the material. However, when the autoclaved extract from the reference method was analyzed by VitaFast, the result was approximately 100% of the expected recovery. This indicates that the enzymatic extraction is not efficacious for the cereal matrix, and the autoclaving preparation is more suitable. For cereal samples, the product insert will be modified to recommend the autoclave extraction method to analyze total biotin content. Although statistical analysis showed a significant difference between the two methods, this is partially due to the low relative standard deviations, or high repeatability of the methods. VitaFast produced results that were within the uncertainty range for the materials tested, with the caveat that the autoclave/acid extraction from the FDA method must be followed for cereal samples that contain predominantly naturally occurring biotin.

Table 7 Intra-assay variance of food samples (triplicate analysis per sample dilution) (1)

Sample description	Expected Value (Label claim) µg/100g	Dilution factor	Mean result in µg / 100 g	Mean result of dilutions In µg / 100 g	Coefficient of variation in %
Vitamin pills	200	10000	192	194	1.3
		20000	194		
		40000	197		
Instant soup	40	360	60	61	1.8
		180	61		
		90	63		
Sausages	120	350	110	117	5.8
		250	118		
		200	122		
English gums	180	400	226	228	1.0
		800	228		
		1600	231		

Table 8 Intra-assay variance of liquid samples (1)

Sample description	Concentration indicated on label (µg / 100 ml)	Dilution factor	Mean result (µg / 100 ml)	Mean result of dilutions (µg / 100 ml)	Coefficient of variation (%)
Beer fruit drink	60	160	79	78	1.2
			78		
		320	77		
			79		
Multivitamin juice	75	75	77	76	1.3
			77		
		150	76		
			75		
12-fruit juice	23	30	28	28	2.1
			27		
		60	28		
			27		

Table 15: Biotin content of two reference materials as determined by two methods, FDA 300/310, and VitaFast P1003. (1)

Sample	NIST 1849 Adult/Infant Nutritional Formula (µg/100g)			AACC VMP-4 2008 Cereal Check Sample (µg/100g)		
	FDA	VitaFast	FDA Extract analyzed by VitaFast	FDA	VitaFast	FDA Extract analyzed by VitaFast
1	188	180.28	130.33	13.2	3.60	10.83
2	181	167.28	139.44	13.2	3.35	10.73
3	185	169.21	120.61	13.8	3.58	10.39
4	190	158.87	142.86	13.4	3.36	9.97
5	187	163.87	131.91	13.8	3.48	10.45
6	184	172.79	131.90	13.6	3.58	10.59
7	192	170.87	140.29	13.6	3.38	10.79
8	190	172.80	132.14	13.5	3.59	10.86
Mean	187.13	169.50	133.68	13.51	3.49	10.58
SD	3.6425	6.4327	7.0903	0.2357	0.1112	0.3016
RSD	1.95	3.79	5.30	1.74	3.19	2.84
Label Value	192 µg/100g (range 167-217 µg/100g)			10.2 µg/100g (range 6.8-14.3 µg/100g)		

Table 16: Statistical Analysis of the results of a method comparison between FDA method 300/310 and VitaFast P1003 (1)

	NIST 1849		**AACC VMP4-2008**	
Two sample F-Test for variances				
	FDA	**VitaFast**	**FDA**	**VitaFast**
Mean	187.13	169.50	13.51	10.58
Variance	13.26786	41.35794	0.055536	0.09037
Observations	8	8	8	8
degrees of freedom (df)	7	7	7	7
F	3.117153		1.627235	
P (F≤f)	0.078434		0.268075	
Critical F value	3.787044		3.787044	
Result	Not significant, use 2 sample t-test with equal variances		Not significant, use 2 sample t-test with equal variances	
	Two sample t-test with unequal variances		**Two sample t-test with equal variances**	
Pooled variance	27.3129		0.072953	
degrees of freedom (df)	14		14	
t-statistic	-6.74633		-21.7421	
P (T≤t) one-tail	4.69e-06		1.73e-12	
Critical t value (one-tail)	1.76131		1.76131	
P (T≤t) two-tail	9.37e-06		3.46e-12	
Critical t value (two tail)	2.144787		2.144787	
Result	Significant difference detected		Significant difference detected	

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