



# CERTIFICATION

**AOAC<sup>®</sup> Performance Tested<sup>SM</sup>**

Certificate No.

**100902**

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

## **VitaFast<sup>®</sup> Vitamin B2 (Riboflavin) Microbiological Microtiter Plate Test for the Determination of Vitamin B2 Riboflavin**

manufactured by  
**ifp Institut für Produktqualität GmbH**  
**Wagner-Régeny-Str. 8**  
**12489 Berlin**  
**Germany**

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

This method has been evaluated in the AOAC<sup>®</sup> *Performance Tested Methods*<sup>SM</sup> Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC<sup>®</sup> Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*<sup>SM</sup> 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 (November 15, 2021 – December 31, 2022). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

*Scott Coates*

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

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November 15, 2021

Date

<b>METHOD AUTHORS</b> Jessica Kerr and Kurt Johnson	<b>SUBMITTING COMPANY</b> R-Biopharm Inc. 7950 Old US 27 South Marshall, MI 49068	<b>Current Sponsor</b> R-Biopharm AG An der neuen Bergstraße 17 64297 Darmstadt Germany
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<b>METHOD NAME</b> VitaFast® Vitamin B2 (Riboflavin) Microbiological Microtiter Plate Test for the Determination of Vitamin B2 Riboflavin	<b>CATALOG NUMBERS</b> P1007
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<b>INDEPENDENT LABORATORY</b> University of Guelph Laboratory Services Division 95 Stone Road West Guelph, ON N1G 2Z4 Canada	<b>AOAC EXPERTS AND PEER REVIEWERS</b> Sneh Bhandari <sup>1,3</sup> , Michael Rychlik <sup>2</sup> <sup>1</sup> Silliker Laboratories, Homewood, IL, USA <sup>2</sup> Technische Universität München, Germany <sup>3</sup> Modification March 2017 (9)
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**APPLICABILITY OF METHOD**

Target analyte – B vitamin riboflavin

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

Performance claims - The performance characteristics of VitaFast® Riboflavin 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 0.0018mg / 100 g as measured by 10 blank samples from 10 different lots. LOQ was set at 0.04mg Riboflavin / 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.

<b>ORIGINAL CERTIFICATION DATE</b> October 28, 2009	<b>CERTIFICATION RENEWAL RECORD</b> Renewed annually through December 2022.
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<b>METHOD MODIFICATION RECORD</b> 1. March 2017 Level 2	<b>SUMMARY OF MODIFICATION</b> 1. Location change to Wagner-Régeny-Str. 8, 12489 Berlin.
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Under this AOAC® <i>Performance Tested</i> <sup>SM</sup> License Number, 100902 this method is distributed by: R-Biopharm AG	Under this AOAC® <i>Performance Tested</i> <sup>SM</sup> License Number, 100902 this method is distributed as: VitaFast® Vitamin B2 (Riboflavin) Microbiological Microtiter Plate Test for the Determination of Vitamin B2 Riboflavin
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**PRINCIPLE OF THE METHOD (1)**

Riboflavin is extracted from the sample and the extract is diluted. The diluted extract and the Riboflavin assay - medium are pipetted into the wells of a microtiter plate which are coated with *Lactobacillus rhamnosus*. The growth of *L. rhamnosus* is dependent on the supply of Riboflavin. Following the addition of Riboflavin 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 Riboflavin 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® Riboflavin test kit is calibrated according to a standard curve of five standard concentrations, using 4-parameter fitting software. The curves shown in figures 1 and 4 are 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. Not only was the result for the AACC reference material consistent across the four lots with a CV of less than 5 %, 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. Recovery of the target concentration was always within 80 - 120 %.

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.

In the independent laboratory study, the VitaFast method agreed well with the reference method for the pig liver sample, although both methods produced results outside the range of uncertainty for this sample, with approximately 120% recovery of the indicated riboflavin content.

Results of the independent study showed that the VitaFast method underestimated the riboflavin content of a commercial cereal sample, when compared to the result from the reference method 940.33. When the autoclaved extract from the reference method was analyzed on the VitaFast microtitre plate, the result agreed with the reference method. This indicates that the difference in the two methods is due only to the extraction efficacy. The autoclave extraction is more suitable for a cereal sample.

**Table 6 Intra-assay variance of food samples (triplicate analysis per sample dilution) (1)**

Sample description (conc. indicated on label in mg / 100 g (ml))	Dilution factor	Mean result in mg / 100 g (ml)	Mean result of dilutions in mg / 100 g (ml)	Coefficient of variation in %
Vitamin pills (4.3 - 5.8)	30	4.30	4.37	1.5
	40	4.43		
	60	4.39		
Multivitamin juice (0.8)	6	0.79	0.78	2.5
	12	0.76		
Cereals (1.3)	7	1.23	1.25	2.6
	14	1.24		
	28	1.29		
Cereal fruit milk powder (0.6)	3	0.61	0.61	1.0
	6	0.60		
	12	0.61		
Fruit pulp (0.7)	20	0.65	0.68	3.7
	40	0.70		
	80	0.68		
	4	0.37		
	8	0.36		

**Table 7 Intra-assay variance of food samples (1)**

Sample description	Labelled Conc (mg/100g(ml))	Mean result (mg/100 g (ml))	Coefficient of variation (%)
Dextrose powder RM – Vit001 internal Reference material	1.6	2.20 (n=6)	1.7
Cornflakes	1.3	1.43 (n=4)	3.3
Choco krispies	1.3	1.04 (n=6)	3.6
Multivitamin juice	0.8	1.13 (n=4)	4.1

**REFERENCES CITED**

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