



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

112102

The AOAC Research Institute hereby certifies the method known as:

RIDASCREEN[®] Peanut

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

distributed by
R-Biopharm Inc.
870 Vossbrink Drive
Washington, MD 63090
USA

This method has been evaluated in the AOAC[®] *Performance Tested MethodsSM* 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 TestedSM* 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 29, 2021 – December 31, 2022). 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 12, 2022
Date

AUTHORS Markus Lacorn, Tina Dubois, Christian Gößwein, Rebecca Kredel, Bianca Ferkinghoff, Sharon Brunelle, Jeremie Theolier, Silvia Dominguez, and Thomas Weiss	SUBMITTING/DISTRIBUTOR COMPANY R-Biopharm Inc. 870 Vossbrink Drive Washington, MD 63090 USA	MANUFACTURED BY R-Biopharm AG An der neuen Bergstraße 17 64297 Darmstadt Germany
METHOD NAME(S) RIDASCREEN® Peanut	CATALOG NUMBERS R6811	
INDEPENDENT LABORATORY Jérémie Théolier ^{a,b} , Silvia Dominguez ^{a,b} ^a PARERA, Institut sur la nutrition et les aliments fonctionnels, Pavillon des services, 2440 Hochelaga, Université Laval, Québec (Québec) G1V 0A6, Canada ^b GForSS, Pavillon Paul Comtois, 2425 Rue De L'Agriculture, Université Laval, Québec (Québec) G1V 0A6, Canada	AOAC EXPERTS AND PEER REVIEWERS Melanie Downs ¹ , Paul Wehling ² ¹ University of Nebraska-Lincoln, Nebraska, USA ² ChemStats Consulting, LLC., Minnesota, USA	
APPLICABILITY OF METHOD Target analyte – Peanut	AOAC SMPR® 2018.012 - Standard Method Performance Requirements (SMPRs®) for Quantitation of Peanut by ELISA-Based Methods. (2)	
<p>Matrices – (1 g samples) Cookies, milk chocolate, trail mix, puffed rice cereal, ice cream, granola bar</p> <p>Performance claims - The results of the independent laboratory evaluation together with the in-house validation study confirmed that RIDASCREEN® Peanut is accurate and suitable to measure peanut in the claimed matrices and meets the requirements laid down in SMPR 2018.012.</p>		
ORIGINAL CERTIFICATION DATE November 29, 2021	CERTIFICATION RENEWAL RECORD New Approval	
METHOD MODIFICATION RECORD NONE	SUMMARY OF MODIFICATION NONE	
Under this AOAC® Performance TestedSM License Number, 112102 this method is distributed by: 1. R-Biopharm Inc.	Under this AOAC® Performance TestedSM License Number, 112102 this method is distributed as: 1. RIDASCREEN® Peanut	

PRINCIPLE OF THE METHOD (1)

The principle of the test is the antigen-antibody reaction. The wells of the microtiter plate are coated with monoclonal antibodies against peanut proteins. By adding the standard or sample solution to the wells, peanut proteins will bind to the specific antibodies. The result is an antibody-antigen complex. Compounds not bound are washed and removed. Then a second monoclonal antibody conjugated to peroxidase (enzyme conjugate) is added. This antibody conjugate binds to the antibody-antigen-complex to form an antibody-antigen-antibody-complex (sandwich). Substrate/chromogen is added after any unbound enzyme conjugate is removed in a washing step. Bound enzyme conjugate converts the chromogen into a blue product. The addition of the stop solution leads to a color change from blue to yellow. The measurement is made photometrically at 450 nm against air. The absorption is proportional to the peanut protein concentration in the sample.

DISCUSSION OF THE VALIDATION STUDY (1)

The sandwich ELISA RIDASCREEN® Peanut (R-Biopharm, R6811) employs the use of two monoclonal antibodies to detect peanut proteins. The extraction method is easy to perform and requires the addition of skim milk powder to all samples. The measurement range is between 0.75 mg/kg and 6 mg/kg peanut commodity using a NIST reference material as calibrator, a dilution of samples up to at least 12 mg/kg peanut is also possible. The ELISA showed no reactivity to 91 different food commodities. LoD was found to be 0.15 mg/kg across three lots and six matrices. LoQ was verified at a level of 0.75 mg/kg. Recoveries studies with incurred milk chocolate and vanilla ice cream revealed consistent mean recoveries between 67 and 85 %. Mean recoveries for incurred cookies depend on the baking temperature and baking time. They ranged from 60 % to 109 %. Spiking of the claimed matrices showed mean recoveries between 65 and 97 % whereas recovery of peanut from trail mix is around 60 %. In most cases, Repeatability (including inhomogeneity of the incurred matrices used) was between 5.2 and 12 %. Depending on the sample type, relative intermediate precision is between 6.4 % and 13.0 %. A ruggedness study revealed no critical parameters for the extraction or ELISA procedure. The test kit is stable for at least 3 weeks at 37 °C and is not susceptible against transport or unintended freezing of the components. The results for incurred cookies and milk chocolate repeated in the independent laboratory showed recoveries between 99 and 104% with RSDs between 3.56 % and 19.5 %. For milk chocolate incurred at a level of 1.00 mg peanut per kg one out of six values was at 0.64 mg/kg and therefore made the RSD raise up to 19.5. Therefore, the independent laboratory was able to confirm the claims of the method developer.

Table 9. Estimation of an overall LoD across six different matrices and three different test kit lots. (1)

Matrix	SD ² (variance), mg/kg
Cookies	0,00166
Milk chocolate	0,00232
Ice cream	0,00213
Trail mix	0,00029
Puffed rice cereals	0,00171
Granola bar	0,00096
Mean variance	0,00151
Mean SD	0,039
LOD (mean + 3.3 x SD)	0,15
LoQ (3 x LOD)	0,45

Table 11. Summary for LoQ data in six different matrices (cookies, milk chocolate, ice cream, trail mix, puffed rice cereal, and granola bar) in three different test kit lots. (1)

Matrix	TC 1		TC 2		TC 3	
	mg/kg	Rec. (%)	mg/kg	Rec. (%)	mg/kg	Rec. (%)
Cookies	0.66	87	0.64	85	0.71	95
Milk chocolate	0.48	69	0.50	66	0.55	78
Ice Cream	0.53	74	0.55	71	0.54	86
Trail mixes	0.38	51	0.41	54	0.50	67
Puffed rice cereal	0.70	94	0.66	88	0.79	106
Granola Bar	0.63	84	0.64	85	0.70	93

Table 25. Summary of residual analysis for each matrix; mean result of concentration across lots per level is given together with the residual in brackets. (1)

Spike mg/kg	Mean result (and residuals) across 3 lots, mg/kg											
	Cookie		Chocolate		Ice cream		Trail mix		Puffed rice cereal		Granola bar	
0,75	0.63	(-0.14)	0.36	(-0.17)	0.58	(-0.09)	0.44	(-0.07)	0.65	(-0.19)	0.63	(-0.09)
1,5	1.24	(-0.14)	0.97	(-0.09)	1.25	(-0.04)	0.93	(-0.03)	1.39	(-0.10)	1.28	(-0.01)
3	2.68	(+0.07)	2.28	(+0.15)	2.54	(+0.01)	1.79	(-0.06)	2.96	(+0.16)	2.46	(+0.02)
6	5.46	(+0.40)	4.52	(+0.25)	5.23	(+0.22)	3.91	(+0.27)	5.71	(+0.28)	4.91	(+0.16)
12	9.77	(-0.19)	8.40	(-0.14)	9.87	(-0.10)	7.08	(-0.11)	10.53	(-0.15)	9.26	(-0.08)

Table 31. Summarized peanut concentrations (mg/kg) of incurred results – Cookies: Independent Laboratory results (1)

Target	Measured ^(a)	Recovery (%)	RSD (%)
1.04	1.04 ± 0.1053	100	10.1
3.12	3.18 ± 0.2679	102	8.42
10.41	10.84 ± 1.1262	104	10.4

^(a) Mean ± standard deviation; n = 6**Table 33. Summarized peanut concentrations (mg/kg) of incurred results – Milk chocolate: Independent Laboratory results (1)**

Target	Measured ^(a)	Recovery (%)	RSD (%)
1.00	0.99 ± 0.1935	99	19.5
3.00	3.02 ± 0.1073	101	3.56
10.00	10.23 ± 1.2437	102	12.2

^(a) Mean ± standard deviation; n = 6**REFERENCES CITED**

1. Lacorn, M. Dubois, Gößwein, C., Kredel, R., Ferkinghoff, B., Brunelle, S., Theolier, J., Dominguez, S., and Weiss, T., Validation of the RIDASCREEN® Peanut for Determination of Peanut Protein in Cookies, Milk Chocolate, Ice Cream, Trail Mix, Puffed Rice Cereals, and Granola Bar, AOAC® Performance TestedSM certification number 112102.
2. AOAC SMPR® 2018.012 - Standard Method Performance Requirements (SMPRs®) for Quantitation of Peanut by ELISA-Based Methods (also available as J. AOAC Int. 102, 326-328, 2019).

