



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

101501

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

RIDASCREEN[®] FAST Milk

manufactured by

R-Biopharm AG

An der neuen Bergstraße 17

D-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 (November 13, 2018 – December 31, 2019). 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

November 13, 2018

Date

METHOD AUTHORS

ORIGINAL VALIDATION: Thomas Weiss, Ulrike Immer, and Markus Lacorn

MODIFICATION MARCH 2017: R-Biopharm

SUBMITTING COMPANY

R-Biopharm AG
An der neuen Bergstraße 17
D-64297 Darmstadt, Germany

KIT NAME(S)

RIDASCREEN®FAST Milk

CATALOG NUMBERS

R4652

INDEPENDENT LABORATORY

Q Laboratories, Inc.
1400 Harrison Ave
Cincinnati, OH
USA

AOAC EXPERTS AND PEER REVIEWERS

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APPLICABILITY OF METHOD

Target organism – two major allergenic proteins in milk: caseins and β -lactoglobulin

Matrices – (1.0 g samples) - cookies, chocolate, ice cream, infant formula and sausages

Performance claims - According to the manufacturer, the RIDASCREEN®FAST Milk detects milk proteins (caseins and β -lactoglobulin) with a mean limit of detection (LOD) of 0.7 mg/kg milk protein. The limit of quantification (LOQ) was set to 2.5 mg/kg milk protein and confirmed by experiments. No cross-reacting substance has been identified by the manufacturer. Ruggedness testing included variation of reagent volume, incubation time and temperature; no significant influence by varying these parameters was observed. Ruggedness testing concerning variation of extraction has not been tested prior to this validation. The lot-to-lot stability testing showed no significant variation between lots. The stability testing regarding shelf life stability included real time stability testing after storage at 4-8°C. Tests showed stable and reproducible test performance during the shelf life of the RIDASCREEN®FAST Milk (maximum shelf life 18 months). Recovery experiments in various matrices showed suitability of RIDASCREEN®FAST Milk for the accurate quantitative detection of milk proteins in at least the following matrices: cookies, sausages, infant formula, ice cream, and chocolate.

ORIGINAL CERTIFICATION DATE

October 13, 2015

CERTIFICATION RENEWAL RECORD

Renewed Annually through December 2019

METHOD MODIFICATION RECORD

1. March 2017

SUMMARY OF MODIFICATION

1. Change to a mercury-free preserving agent in the washing buffer
(2)

Under this AOAC® *Performance Tested*SM License Number, 101501 this method is distributed by:

NONE

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NONE

PRINCIPLE OF THE METHOD (1)

The basis of the test is an antigen-antibody reaction. Specific antibodies against casein and β -lactoglobulin are used for the detection. A microtiter plate is coated with both antibodies as capture antibodies. Skim milk powder calibrators (traceable to NIST whole milk powder RM1549a) and sample extracts are incubated for 10 minutes. After washing, a mixture of the anti-casein and anti- β -lactoglobulin antibody enzyme conjugates is added for further 10 minutes. This conjugate binds to the milk protein – antibody complex on the plate (sandwich enzyme immunoassay). Any unbound enzyme conjugate is then removed by a washing step. Substrate/chromogen is added to the wells and incubated for 10 min. Bound enzyme converts the substrate/chromogen into a blue colored product. The addition of the stop reagent inhibits the enzymatic process and causes a shift of the colored product to yellow. Measurement is performed photometrical within 10 min after stopping at 450 nm against air. The resulting absorbance values are proportional to the concentration of milk protein in a sample.

DISCUSSION OF THE VALIDATION STUDY (1)

All data collected show that RIDASCREEN®FAST Milk is capable of analyzing milk proteins at concentrations starting at 2.5 mg/kg up to 30 mg/kg in processed cookies, chocolate, chocolate desert, ice cream, infant formula and sausages. The LOD of the method is 0.7 mg/kg. The test kit is robust against variation of the test implementation itself as well as sample preparation.

The test kit manufacturer and the external laboratory recommend the method as AOAC Performance Tested MethodSM.

Table 20 Summary of matrix study (1)

Mean values of calculated recoveries and standard deviation of recoveries are shown. Overall mean has been calculated from the mean values of each spiking level of each matrix.

	Spike level (mg/kg)	Mean concentration (mg/kg)	Standard deviation repeat-ability (s_r)	Rel. standard deviation repeatability (RSD _r in %)	Mean recovery (%)	Standard deviation recovery (%)
Cookies	milk free	< LOQ				
	5	4.9	0.48	9.7	98.7	9.6
	10	11.6	1.1	9.6	115.9	11.2
	30	33.6	3.2	9.6	112.0	10.8
Infant formula	milk free	< LOQ				
	5	5.0	0.50	10.0	99.6	9.9
	10	10.4	0.69	6.6	104.3	6.9
	30	28.0	2.8	10.1	93.4	9.5
Chocolate	milk free	< LOQ				
	5	5.7	0.30	5.3	112.9	5.3
	10	12.8	0.88	6.9	127.7	6.9
	30	32.4	1.0	3.1	107.8	3.1
Ice cream	milk free	< LOQ				
	5	4.9	0.21	4.4	97.0	4.3
	10	11.2	0.19	1.7	112.4	1.9
	30	30.8	0.88	2.9	102.6	2.9
Sausage	milk free	< LOQ				
	5	4.7	0.35	7.4	94.0	6.9
	10	10.2	0.68	6.7	101.7	6.8
	30	29.1	1.05	3.6	97.0	3.5
Mean all four matrices milk free		< LOQ				
Mean all four matrices + 5 mg/kg		5.0	0.36	7.2	100.4	7.2
Mean all four matrices + 10 mg/kg		11.2	0.75	6.7	112.4	6.7
Mean all four matrices + 30 mg/kg		30.8	1.83	6.0	102.6	6.0
Overall mean (all spiking levels of all four matrices)					105.1	6.6

REFERENCES CITED

1. Weiss, T., Immer, U., and Lacorn, M., Evaluation of the R-Biopharm RIDASCREEN®FAST Milk, AOAC® *Performance Tested*SM certification number 101501.
2. R-Biopharm, Evaluation of Requested exchange of thimerosal in RIDASCREEN®FAST Milk R4652, AOAC® *Performance Tested*SM certification number 101501. Approved March 2017