



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
101501

The AOAC Research Institute hereby certifies the method 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 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.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date

February 2, 2023

Expiration Date

December 31, 2023

METHOD AUTHORS ORIGINAL VALIDATION: Thomas Weiss, Ulrke Immer, and Markus Lacorn MODIFICATION MARCH 2017: R-Biopharm	SUBMITTING COMPANY R-Biopharm AG An der neuen Bergstraße 17 D-64297 Darmstadt, Germany
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KIT NAME(S) RIDASCREEN®FAST Milk	CATALOG NUMBERS R4652
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INDEPENDENT LABORATORY Q Laboratories, Inc. 1400 Harrison Ave Cincinnati, OH USA	AOAC EXPERTS AND PEER REVIEWERS Bert Popping ¹ , Eric Garber ² , Sarwar Gilani ³ , Sneha Bhandari ^{4, 5} ¹ Mérieux NutriSciences, FRANCE ² Food and Drug Administration, Center for Food Safety and Applied Nutrition, Maryland, USA ³ FAOACI, FFPAS, Ontario, CANADA ⁴ Merieux Nutri Sciences, Illinois, USA ⁵ Modification March 2017
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APPLICABILITY OF METHOD
Target organism – two major allergenic proteins in milk: caseins and β -lactoglobulin.

Matrixes – (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 matrixes showed suitability of RIDASCREEN®FAST Milk for the accurate quantitative detection of milk proteins in at least the following matrixes: cookies, sausages, infant formula, ice cream, and chocolate.

ORIGINAL CERTIFICATION DATE October 13, 2015	CERTIFICATION RENEWAL RECORD Renewed annually through December 2023.
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METHOD MODIFICATION RECORD 1. March 2017 Level 2 2. December 2021 Level 1	SUMMARY OF MODIFICATION 1. Change to a mercury-free preservative in the washing buffer (2) 2. Editorial changes
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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 matrixes milk free		< LOQ				
Mean all four matrixes + 5 mg/kg		5.0	0.36	7.2	100.4	7.2
Mean all four matrixes + 10 mg/kg		11.2	0.75	6.7	112.4	6.7
Mean all four matrixes + 30 mg/kg		30.8	1.83	6.0	102.6	6.0
Overall mean (all spiking levels of all four matrixes)					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 MethodsSM certification number 101501.
2. R-Biopharm, Evaluation of Requested exchange of thimerosal in RIDASCREEN®FAST Milk R4652, AOAC Performance Tested MethodsSM certification number 101501. Approved March 2017