



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
091701

The AOAC Research Institute hereby certifies the method known as:

Milk Protein Rapid Kit (Casein)

manufactured by

Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912
USA

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*SM Program. 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, appearing to read "Bradley A. Stawick", written over a horizontal line.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date
Expiration Date

December 06, 2024
December 31, 2026

AUTHORS ORIGINAL VALIDATION: Luke Emerson-Mason, Raquel Sobel, Adam Bouchard, and Thomas Grace MODIFICATION FEBRUARY 2022: Karen Silbernagel MODIFICATION SEPTEMBER 2022: April Schumacher		SUBMITTING COMPANY Elution Technologies 480 Hercules Drive Colchester, VT 05446	CURRENT SPONSOR Neogen Food Safety Corporation 620 Leshner Place Lansing, MI 48912
METHOD NAME Neogen® Milk Protein Rapid Kit (Casein) *Formerly known as 3M™ Milk Protein Rapid Kit (Casein) and 3M™ Bovine Total Milk Protein Rapid Kit and Elution Technologies Milk Rapid Test Kit		CATALOG NUMBER L25MLK	
INDEPENDENT LABORATORY Q Laboratories 1400 Harrison Ave. Cincinnati, OH 45214 USA			
APPLICABILITY OF METHOD Target analytes – Bovine Milk Proteins. Matrixes – soy milk (100 µL), baked sugar cookies (0.2 g), chocolate dessert (0.2 g), clean in place solution (200 µL), stainless steel (100 cm²) Performance claims – The Milk Protein Rapid Kit Casein utilizes the ICL antibody to detect proteins from bovine milk. The LOD for food products, including finished products and CIP, is 2 ppm milk protein, or 2.5 µg protein per ml per 100 cm² swabbed surface area.		REFERENCE MATERIAL NIST SRM 1549a Whole Milk Powder, protein content 25.64% by certificate of analysis	
ORIGINAL CERTIFICATION DATE September 6, 2017		CERTIFICATION RENEWAL RECORD Renewed through December 2026.	
METHOD MODIFICATION RECORD 1. March 2018 Level 1 2. January 2019 Level 1 3. December 2019 Level 1 4. December 2020 Level 1 5. November 2021 Level 1 6. February 2022 Level 2 7. September 2022 Level 2 8. January 2024 Level 1		SUMMARY OF MODIFICATION 1. Sponsor change from Elution Technologies to 3M. Editorial changes to insert to reflect the change. Kit name updated to 3M™ Bovine Total Milk Protein Rapid Kit from Milk Rapid Test. 2. Revision of kit name and editorial changes to insert. 3. Editorial/clerical changes. Change of name of IFU Bovine replaced with Milk. 4. Addition of two general warning statements regarding allergens. 5. Editorial changes. 6. Manufacturing location change. 7. Manufacturing location change for bottling of buffer solutions and kit assembly. 8. Editorial changes to rebrand method from 3M to Neogen Corporation.	
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PRINCIPLE OF THE METHOD (1)

The Neogen® Milk Protein Rapid Kit Casein is a rapid immunochromatographic lateral flow test device which utilizes a purified proprietary poly-clonal antibody developed by ICL against proteins from bovine milk.

DISCUSSION OF THE VALIDATION STUDY (1)*Food Matrix Study*

Soy milk and chocolate dessert matrix were inoculated as described in the methods section, and analyzed at 5 levels (0 ppm, 1-2 ppm, 3 ppm, 5 ppm and 10 ppm). Results for both the reference method and LFDs are presented in Table 5, with calculated PODs and 95% Confidence Intervals.

All unspiked samples were negative at 11 minutes, with PODs of 0.00. All samples spiked at 3 ppm, 5 ppm, and 10 ppm were positive at 11 minutes, with PODs of 0.97-1.00. POD values for soy milk spiked at 1.25 ppm and chocolate dessert matrix spiked at 1.5ppm were fractionally positive with PODs of 0.57 and 0.50, respectively.

Soy Milk spiked at 10,000 ppm with NIST SRM 1549a was also tested to determine if there was an overload, or “hook” effect at high levels of contamination. As shown in Table 5, the tests were invalid as the Hook and Test Lines failed to appear due to the high concentration of milk protein, although the Control Line was still present.

Incurred Samples Study

As seen in Table 5, PODs for the negative cookie samples were 0.00 at 11 minutes; PODs for the 5 and 10 ppm samples were 1.00 at 11 minutes. Only 4 out of 30 sample incurred at 1 ppm were positive; 18 of 30 samples incurred at 3ppm were positive, with a fractional POD of 0.60. The limit of detection in incurred samples is therefore determined to be 3 ppm.

Environmental Surface Testing Study

Clean in Place (CIP) rinse water, pH 12.7 was tested at 0 ppm, 2 ppm, 3 ppm, 5 ppm and 10 ppm NIST-spiked concentrations with n=30 replicates per concentration by the candidate method, as well as n=3 replicates per concentration by the reference method. Results are shown in Table 6 and Figure 4, with POD values of 0.00 for all unspiked replicates, and PODs of 1.00 for all 3, 5 and 10 ppm replicates at 11 minutes. Additionally, stainless steel was tested on the candidate method by swabbing 100 cm² surfaces coated with 1 ml each of either blank PBS (n=5), 1.25 ug/ml NIST milk protein spike (n=30), or 2.5 ug/ml NIST milk protein spike (n=5), and allowed to dry overnight. Tables 6 and 7 show results from both the sponsor laboratory and independent laboratory for stainless steel surface testing; both laboratories found PODs of 0.00 for 0 ug/ml/100 cm² and 1.00 at 11 min for 2.5 ug/ml/100 cm². At 1.25 ug/ml the sponsor lab found a POD of 0.43 at 11 minutes, while the independent lab found a POD of 0.67 at 11 minutes. Both laboratories reported good correlation between POD values for each inoculation level.

Table 1. Food Matrix Testing (1)

Matrix	Spike concentration ppm	Number of replicates	Number of positive results at 11min	POD at 11 min	95% CI at 11 min	Average results from AOACRI PTM 1101501, n=3
Soy Milk	0	30	0	0.00	0.00, 0.11	<2.5*
	1.25	30	17	0.57	0.39, 0.73	3.10
	3	30	29	0.97	0.83, 1.00	4.42
	5	30	29	0.97	0.83, 1.00	5.95
	30	30	30	1.00	0.89, 1.00	10.97
	10,000	10	No Hook Lines	N/A	N/A	N/A
Chocolate Dessert	0	30	0	0.00	0.00, 0.11	<2.5*
	1.5	30	15	0.5	0.33, 0.67	<2.5*
	3	30	29	0.97	0.83, 1.00	<2.5*
	5	30	30	1	0.89, 1.00	5.80
	30	30	30	1	0.89, 1.00	11.90
Baked Cookies	0	30	0	0.00	0.00, 0.11	<2.5*
	1	30	4	0.13	0.05, 0.30	<2.5*
	3	30	18	0.6	0.42, 0.75	3.27
	5	30	30	1	0.89, 1.00	5.77
	10	30	30	1	0.89, 1.00	11.2

*LOQ of the assay is 2.5ppm; result may not be significant

Table 2. Surface Testing

Matrix	Spike concentration ppm	Number of replicates	Number of positive results at 11min	POD at 11 min	95% CI at 11 min	Average results from AOACRI PTM 1101501, n=3
CIP	0	30	0	0.00	0.00, 0.11	<2.5*
	2	30	19	0.63	0.46, 0.78	<2.5*
	3	30	30	1	0.89, 1.00	2.51
	5	30	30	1	0.89, 1.00	5.99
	10	30	30	1	0.89, 1.00	11.17
	Spike concentration ug/ml/100 cm ²	Number of replicates	Number of positive results at 11min	POD at 11 min	95% CI at 11 min	Average results from AOACRI PTM 1101501, n=3
Swabbing	0	5	0	0.00	0.00, 0.43	N/A
	1.25	30	13	0.43	0.27, 0.61	N/A
	2.50	5	5	1.00	0.57, 1.00	N/A

*LOQ of the assay is 2.5ppm; result may not be significant

REFERENCES CITED

- Emerson-Mason, L., Sobel, R., Bouchard, A., and Grace, T., Evaluation of the 3M™ Milk Protein Rapid Kit Casein (formerly Elution Technologies Milk Rapid Test) for the Detection of Total Milk Proteins in Foods and on Surfaces, AOAC Performance Tested MethodsSM certification number 091701.
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- Asthma and Allergy Foundation of America- <http://www.kidswithfoodallergies.org/page/milk-allergy.aspx> (Accessed December 2016)
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<http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Allergens/ucm106187.htm> (Accessed January 2017).
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- Schumacher, A., AOAC Performance Tested MethodSM Level 2 Modification 3M™ Milk Protein Rapid Kit (Casein) - PTM 091701, 3M™ Coconut Protein Rapid Kit - PTM 061903, 3M™ Gluten Protein Rapid Kit - PTM 011601, and 3M® Egg White Protein Rapid Kit – PTM 052001, Certification number 011601. Approved September 16, 2022.