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.

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

Issue Date	December 06, 2024
Expiration Date	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 Lesher 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	SUMMARY OF MODIFICATION
1. March 2018 Level 1	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. January 2019 Level 1	2. Revision of kit name and editorial changes to insert.
3. December 2019 Level 1	3. Editorial/clerical changes. Change of name of IFU Bovine replaced with Milk.
4. December 2020 Level 1	4. Addition of two general warning statements regarding allergens.
5. November 2021 Level 1	5. Editorial changes.
6. February 2022 Level 2	6. Manufacturing location change.
7. September 2022 Level 2	7. Manufacturing location change for bottling of buffer solutions and kit assembly.
8. January 2024 Level 1	8. Editorial changes to rebrand method from 3M to Neogen Corporation.

Under this AOAC *Performance Tested Methods℠* License Number, 091701 this method is distributed by:
NONE

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NONE

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 cm^2 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 cm^2 and 1.00 at 11 min for 2.5 ug/ml/100 cm^2. 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 cm²	**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

1. 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 Methods℠* certification number 091701.
2. AOAC Research Institute Validation Outline for 3M® Milk Protein Rapid Kit Casein (formerly Elution Technologies Milk Rapid Test for the Detection of Total Milk Proteins in Foods and on Surfaces.
3. Fiocchi A, Brozek J, Schunemann HJ, Bahna SL, von Berg A, Beyer K, et al. (2010) *World Allergy Organization Journal* 3, 57-61
4. Asthma and Allergy Foundation of America- http://www.kidswithfoodallergies.org/page/milk-allergy.aspx (Accessed December 2016)
5. U.S. FDA Food and Allergen Labeling and Consumer Protection Act of 2004 http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Allergens/ucm106187.htm (Accessed January 2017).
6. AOAC International Stake Holders Panel on Alternative Methods Guidelines for Validation of Qualitative Binary Chemistry Methods 19th Ed., Appendix N AOAC INTERNATIONAL, Gaithersburg, MD, http://www.eoma.aoac.org (Accessed January 2017).
7. Schumacher, A., AOAC *Performance Tested Method℠* 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.