CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

091701

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

3M™ Milk Protein Rapid Kit Casein

manufactured by

3M
Food Safety Department
3M Center, Bldg. 275-5W-05
St. Paul, MN 55144

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 (December 13, 2019 – December 31, 2020). 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

December 13, 2019
Date

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CURRENT SPONSOR
3M
Food Safety Department
3M Center, Bldg. 275-5W-05
St. Paul, MN 55144

KIT NAME(S)
3M™ Milk Protein Rapid Kit Casein
Formerly known as 3M™ Bovine Total Milk Protein Rapid Kit

CATALOG NUMBERS
L25MLK

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APPLICABILITY OF METHOD
Target analytes – Bovine Milk Proteins

Matrices – soy milk, baked sugar cookies, chocolate dessert, clean in place solution, stainless steel (1 x 1 in swab)

Performance claims - The Elution Technologies Milk Rapid Test Kit 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 cm^2 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 annually through December 2020

METHOD MODIFICATION RECORD
1. **March 2018 Level 1**
2. **January 2019 Level 1**
3. **December 2019 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.**

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

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

PRINCIPLE OF THE METHOD (1)
The Elution Technologies Milk Rapid Test Kit 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 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

1. Emerson-Mason, L., Sobel, R., Bouchard, A., and Grace, T., Evaluation of the Elution Technologies Milk Rapid Test for the Detection of Total Milk Proteins in Foods and on Surfaces, AOAC® *Performance Tested*℠ certification number 091701.
2. AOAC Research Institute Validation Outline for 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).