



# CERTIFICATION

**AOAC<sup>®</sup> Performance Tested<sup>SM</sup>**

Certificate No.

**061903**

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

**3M<sup>™</sup> Coconut Protein Rapid Kit**

manufactured by

**3M**

**Food Safety Department**

**3M Center, Bldg 275-5W-05**

**St. Paul, MN 55144**

This method has been evaluated in the AOAC<sup>®</sup> *Performance Tested Methods*<sup>SM</sup> Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC<sup>®</sup> Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*<sup>SM</sup> 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 08, 2020 – December 31, 2021). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

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

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

\_\_\_\_\_  
December 08, 2020

Date

**METHOD AUTHORS**

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**SUBMITTING COMPANY**3M  
Food Safety Department  
3M Center, Bldg 275-5W-05  
St. Paul, MN 55144**KIT NAME(S)**

3M™ Coconut Protein Rapid Kit

**CATALOG NUMBERS**

L25C0C

**INDEPENDENT LABORATORY**NSF International – Applied Research Center  
789 N. Dixboro Rd  
Ann Arbor, MI 48015**AOAC EXPERTS AND PEER REVIEWERS**Qinchun Rao<sup>1</sup>, Joe Boison<sup>2</sup>, Melanie Downs<sup>3</sup>  
<sup>1</sup> Florida State University, Tallahassee, FL, USA  
<sup>2</sup> Consultant, Saskatoon, Canada  
<sup>3</sup> Department of Food Science & Technology, University of Nebraska – Lincoln, USA**APPLICABILITY OF METHOD**

Target analyte – Coconut proteins

Matrixes – (solid samples, 0.2 g) - blueberry yogurt, chocolate powder, incurred cookie  
(liquid samples, 100 µL) - soy milk, vanilla ice cream  
(200 µL) - clean-in-place (CIP) final rinse water  
(10 x 10 cm) - environmental swab stainless steel

Performance claims - The sensitivity of the 3M Coconut Protein Rapid Kit is intended for screening the presence of coconut proteins at 2 ppm for clean-in-place (CIP) fluids, environmental surfaces and select food matrixes.

**ORIGINAL CERTIFICATION DATE**

June 14, 2019

**CERTIFICATION RENEWAL RECORD**

Renewed annually through December 2021

**METHOD MODIFICATION RECORD**

1. December 2020 Level 1

**SUMMARY OF MODIFICATION**

1. Addition of 2 general warning statements regarding allergens.

Under this AOAC® *Performance Tested*<sup>SM</sup> License Number, 061903 this method is distributed by:  
NONE

Under this AOAC® *Performance Tested*<sup>SM</sup> License Number, 061903 this method is distributed as:  
NONE

**PRINCIPLE OF THE METHOD (1)**

This lateral flow assay is an immunochromatographic method utilizing polyclonal antibodies for the specific capture and recognition of coconut protein. Protein is extracted from the matrix of interest and loaded into the sample well, from which it is pulled through a series of membranes by capillary action. The sample interacts with antibody conjugated with gold nanoparticles (conjugate), then flows through a nitrocellulose membrane striped with a test, hook, and control line. The test line captures coconut proteins in the sample, forming a sandwich with conjugated antibody and thus facilitating color development in the presence of coconut proteins. The hook line is a competitive binding assay used as a control to determine if a sample contains an excess of coconut protein that could quench the test line. Finally, the control line contains a second antibody to capture conjugated antibody and verify that the test flowed as intended. This assay is intended for screening of CIP final rinse water, swabs, raw ingredients and finished product for the presence of coconut protein down to 2 ppm (sensitivity may vary depending on matrix). Samples that contain more than 5% coconut proteins may result in an invalid test.

**DISCUSSION OF THE VALIDATION STUDY (1)**

This study demonstrated that the 3M Coconut Protein Rapid Kit can detect coconut proteins in select foods and environmental surfaces without cross reactivity or interference; coconut proteins were not detected in un-spiked samples while it was detected in a variety of foods spiked with 10 ppm of coconut proteins.

The generation of probability of detection curves showed a dose response for all matrixes tested including food samples, stainless steel coupons and swabs utilized for environmental monitoring. In the matrix study it was possible to achieve fractional results (25–75% of positive samples) in spiked test samples that ranged from 0.20 to approximately 1.25 ppm depending on matrix. A probability of detection of 1.0 at levels that ranged between 1 and 2 ppm was achieved in most matrixes. In the case of spiked vanilla ice cream, POD data was provided by the independent laboratory and the method developer (Figures 5 and 6). It was not possible to observe a dose response effect while conducting the evaluation at the independent laboratory. The highest concentrations tested between the third-party lab and the method developer were 1 and 2 ppm, respectively. Discrepant POD values were observed to have a ten-fold difference, POD of 0.10 for the independent lab and POD of 1.00 for the method developer. The results from the independent laboratory reported the presence of a faint test line which supports the possibility of incorrect preparation of standards. The observance of POD of 1.00 at concentrations 1 ppm and 2 ppm from the method developer for the spiked vanilla ice cream indicate that the method is capable of detecting coconut proteins if present.

All matrixes were pre-screened by both the 3M Coconut Protein ELISA Kit and r-Biopharm® Coconut Lateral Flow Assay. Results were negative for all matrixes except CIP which was provided by industry; the 3M Coconut Protein ELISA Kit indicated there was less than 2 ppm of coconut proteins present in the sample while the r-Biopharm® Coconut Lateral Flow Assay did not detect coconut in this sample. This discrepancy may be due to kit design, sensitivity and limit of detection differences between the kits. Pre-screening with the r-Biopharm® Coconut Lateral Flow Assay may not be appropriate due to the lack of instructions for clean-in-place rinse water.

The Coconut Protein Rapid Kit is lot specific i.e. components are not interchangeable between lots. Results among different lots of products showed the same response in all lots tested indicating that the product delivered consistent results among different lots throughout the claimed shelf life of 24 months.

Similarly, robustness study showed that variations in temperature of the lateral flow device, incubation time and volume of extracted sample added to the LFD did not affect the detection of coconut proteins.

**Table 5. Matrix Study Evaluation at Various concentrations using the 3M Coconut Protein Rapid Kit (1)**

Matrix	Spike level	Targeted concentration (ppm)	Candidate				
			N	x	POD <sub>c</sub> <sup>a</sup>	95% LCL <sup>b</sup>	95% UCL <sup>c</sup>
Incurred cookie	L(0)	0	30	0	0.00	0.00	0.11
	L(1)	0.5	30	18	0.60	0.42	0.75
	L(2)	1.0	30	21	0.70	0.52	0.83
	L(3)	1.5	30	30	1.00	0.89	1.00
Blueberry yogurt	L(0)	0	30	0	0.00	0.00	0.11
	L(1)	0.75	30	6	0.20	0.10	0.37
	L(2)	0.85	30	9	0.30	0.17	0.48
	L(3)	1.00	30	30	1.00	0.89	1.00
	L(4)	1.25	30	30	1.00	0.89	1.00
	L(5)	2.00	30	30	1.00	0.89	1.00
	L(6)	5.00	30	30	1.00	0.89	1.00
Chocolate Powder	L(0)	0	30	0	0.00	0.00	0.11
	L(1)	0.75	30	4	0.13	0.05	0.30
	L(2)	1.00	30	22	0.73	0.56	0.86
	L(3)	1.25	30	13	0.43	0.27	0.61
	L(4)	2.00	30	30	1.00	0.89	1.00
Soy Milk	L(0)	0	30	0	0.00	0.00	0.11
	L(1)	0.25	30	0	0.00	0.00	0.11
	L(2)	0.50	30	5	0.17	0.07	0.34
	L(3)	0.75	30	14	0.47	0.30	0.64
	L(4)	1.0	30	29	0.97	0.83	1.00
Vanilla Ice Cream (Third Party Data)	L(0)	0	30	0	0.00	0.00	0.11
	L(1)	0.25	30	8	0.27	0.14	0.44
	L(2)	0.50	30	0	0.00	0.00	0.11
	L(3)	0.75	30	14	0.47	0.30	0.64
	L(4)	1.00	30	3	0.10	0.03	0.26
Vanilla Ice Cream (3M Internal Data)	L (0)	0	30	0	0.00	0.00	0.11
	L (1)	0.25	30	10	0.33	0.19	0.51
	L (2)	0.30	30	10	0.33	0.19	0.51

	L (3)	0.50	30	21	0.70	0.52	0.83
	L (4)	0.70	30	25	0.83	0.66	0.93
	L (5)	1.0	30	30	1.00	0.89	1.00
	L (6)	2.0	30	30	1.00	0.89	1.00
CIP Final Rinse Water	L (0)	0	30	0	0.00	0.00	0.11
	L (1)	0.15	30	4	0.13	0.05	0.30
	L (2)	0.20	30	20	0.67	0.49	0.81
	L (3)	0.25	30	26	0.87	0.70	0.95
	L (4)	0.50	30	28	0.93	0.79	0.98
	L (5)	0.75	30	27	0.90	0.74	0.97
	L (6)	1.00	30	28	0.93	0.79	0.98
Stainless Steel Surface	L (0)	0	7	0	0.00	0.00	0.35
	L (1)	0.75	30	17	0.57	0.39	0.73
	L (2)	0.90	30	18	0.60	0.42	0.75
	L (3)	1.00	30	24	0.80	0.63	0.90
	L (4)	1.25	30	27	0.9	0.74	0.97
	L (5)	2.00	30	30	1.00	0.89	1.00
	L (6)	3.00	30	30	1.00	0.89	1.00
	L (7)	5.00	5	5	1.00	0.57	1.00

All tested matrixes were pre-screened with r-Biopharm® Coconut Lateral Flow Assay to confirm coconut proteins were not present.

<sup>a</sup>POD (c). Probability of detection for the candidate method result.

<sup>b</sup>LCL. 95% confidence interval lower control limit.

<sup>c</sup>UCL. 95% confidence interval upper control limit.

#### REFERENCES CITED

1. Monteroso, L., Velasco, G.L., and Celt, M., Validation of the Coconut Protein Rapid Kit for the Detection of Coconut Proteins in Select Foods and Environmental Surfaces, AOAC® *Performance Tested<sup>SM</sup>* certification number 061903.
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7. LaBudde, R.A. (2009) Statistical analysis of interlaboratory studies. XX. Measuring the performance of a qualitative test method. AOAC Binary Data Interlaboratory Study Workbook Version 2.3 -<http://lclftd.com/aoac/aoac-binary-v2-2.xls> (Accessed January 2019).