CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

061903

The AOAC Research Institute hereby certifies the method known as:

Coconut Protein Rapid Kit

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 09, 2024
Expiration Date	December 31, 2026

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SUBMITTING COMPANY
3M Company
3M Center, Bldg 275-5W-05
St. Paul, MN 55144

CURRENT SPONSOR
Neogen Food Safety Corporation
620 Lesher Place
Lansing, MI 48912

METHOD NAME
Neogen® Coconut Protein Rapid Kit
formerly 3M™ Coconut Protein Rapid Kit

CATALOG NUMBER
L25COC

INDEPENDENT LABORATORY
NSF International – Applied Research Center
789 N. Dixboro Rd
Ann Arbor, MI 48015 USA

APPLICABILITY OF METHOD
Target analyte – Coconut proteins

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

Performance claims – The sensitivity of the 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 through December 2026.

METHOD MODIFICATION RECORD
1. **December 2020 Level 1**
2. **September 2022 Level 2**
3. **January 2024 Level 1**

SUMMARY OF MODIFICATION
1. **Addition of two general warning statements regarding allergens.**
2. **Manufacturing location change for bottling of buffer solutions and kit assembly.**
3. **Editorial/clerical changes to rebrand method from 3M to Neogen Corporation.**

Under this AOAC *Performance Tested Method*℠ License Number, 061903 this method is distributed by:
NONE

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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 Neogen® 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 Neogen 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 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 Coconut Protein Rapid Kit (1)

Matrix	Spike level	Targeted concentration (ppm)	Candidate				
			N	x	POD$_c$[a]	95% LCL[b]	95% UCL[c]
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.

[a]POD (c). Probability of detection for the candidate method result.

[b]LCL. 95% confidence interval lower control limit.

[c]UCL. 95% confidence interval upper control limit.

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