



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
052001

The AOAC Research Institute hereby certifies the method known as:

Egg White 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.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

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

Issue Date
Expiration Date

December 05, 2024
December 31, 2026

AUTHORS ORIGINAL VALIDATION: April Schumacher and Gabriela Lopez Velasco MODIFICATION SEPTEMBER 2022: April Schumacher		SUBMITTING COMPANY 3M Company 3M Center, Bldg 275-5W-05 St. Paul, MN 55144	CURRENT SPONSOR Neogen Food Safety Corporation 620 Leshar Place Lansing, MI 48912
METHOD NAME Neogen® Egg White Protein Rapid Kit Formerly 3M™ Egg White Protein Rapid Kit		CATALOG NUMBER L25EGG	
INDEPENDENT LABORATORY Q Laboratories Cincinnati, OH 45204 USA		APPLICABILITY OF METHOD Target analyte – Egg White Protein. Matrixes – vanilla ice cream (100 µL), whole fluid milk (100 µL), orange juice (100 µL), salad dressing (100 µL), Liquid chocolate syrup (500 µL), incurred cookie (0.2 g), clean-in-place (CIP) final rinse water (200 µL), environmental swab on stainless steel (10 x 10 cm surface area) Performance claims – The sensitivity of the Egg White Proteins Rapid Kit is intended for detecting the presence of total egg protein at 1 ppm for clean-in-place (CIP) fluids, environmental surfaces and select food matrixes.	
ORIGINAL CERTIFICATION DATE May 08, 2020		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 AOAC approval statement. Change to LOD from 0.5 to 0.6 ppm because of the AOAC validation study. Addition of two statements regarding the effect of processing and requirements for user regarding fit for purpose. 2. Manufacturing location change for bottling of buffer solutions and kit assembly. 3. Editorial changes to rebrand method from 3M to Neogen Corporation.	
Under this AOAC <i>Performance Tested Methods</i> SM License Number, 052001 this method is distributed by: NONE		Under this AOAC <i>Performance Tested Methods</i> SM License Number, 052001 this method is distributed as: NONE	

PRINCIPLE OF THE METHOD (1)

The Neogen® Egg White Protein Rapid Kit is an immunochromatographic method that utilizes a lateral flow device containing polyclonal antibodies for the specific capture and recognition of egg white proteins. 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 egg white proteins in the sample, forming a sandwich with conjugated antibody and thus facilitating color development in the presence of egg white proteins. The hook line is a competitive binding assay used as a control to determine if a sample contains an excess of egg white 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. The test can be interpreted as a positive result when three lines appear on the LFD, one at the test line, another at the hook line, and one at the control line. The Egg White Protein Rapid Kit is intended to be used for screening clean-in-place (CIP) final rinse water, environmental swab samples, food ingredients and processed food products for the presence of egg white protein at levels of 1 ppm to approximately 10,000 ppm depending on the matrix being tested. Samples that contain more than 10,000 ppm egg protein may result in an invalid test.

DISCUSSION OF THE VALIDATION STUDY (1)

This study demonstrated that the Egg White Protein Rapid Kit can detect egg proteins in select foods without cross reactivity or interference. Egg proteins were not detected in un-spiked samples while they were detected in a variety of foods spiked with 2 ppm of egg protein utilizing the NIST 8445 standard. The generation of probability of detection curves showed a dose response for all matrixes tested including food samples, CIP final rinse water, 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 0.25 to 0.5 ppm depending on matrix. A probability of detection of 1.0 at 1 ppm was achieved for all matrixes except for liquid chocolate syrup. In chocolate syrup a probability of 0.93 and 0.97 was obtained in samples spiked with 1 or 2 ppm of the egg NIST 8445 standard respectively. This corresponds to one negative result out of 30 spiked samples at 2 ppm and two negative results out of 30 samples spiked at 1 ppm, thus it is unlikely that this is associated with matrix interference. It should be considered that chocolate syrup is a thick matrix and samples were individually spiked, thus homogenization of the spike into the matrix for extraction may have impacted the outcome. The Egg White Protein Rapid Kit product instructions indicate an alternative protocol for ‘chocolate samples’ that requires additional dilutions which may impact the limit of detection and could contribute to the results obtained in the matrix study. All matrixes were pre-screened by both the Neogen Egg White Protein ELISA Kit and Romer AgraStrip® Whole Egg lateral flow assay. Results were negative for all matrixes except chicken; the Egg White Protein ELISA Kit indicated there was less than 0.5 ppm of egg white protein, however an optical density larger than the blank sample was obtained, similarly the Romer AgraStrip® Whole Egg lateral flow assay detected egg in this sample. The possible cross-reactivity with the pre-screening methods may be associated to the kit design, sensitivity and limit of detection.

Table 4. Selectivity study to assess cross reactivity and interference of the Egg White Protein Rapid Kit with other food commodities (1)

Commodity Tested ^a	Screening of non-spiked samples (Cross reactivity)		Screening of spiked sample at 2 ppm of egg protein(interference)	
	N ^b	Result	N ^b	Result
Buckwheat	1	No detection	1	Positive
Cocoa	1	No detection	1	Positive
Lecithin	1	No detection	1	Positive
Whole milk	1	No detection	1	Positive
Pumpkin seed	1	No detection	1	Positive
Oats	1	No detection	1	Positive
Coconut	1	No detection	1	Positive
Gelatin (bovine)	1	No detection	1	Positive
Lentils	1	No detection	1	Positive
Sunflower seed	1	No detection	1	Positive
Corn	1	No detection	1	Positive
Hazelnut	1	No detection	1	Positive
Peanut	1	No detection	1	Positive
Sesame	1	No detection	1	Positive
Chickpeas	1	No detection	1	Positive
Rice flour	1	No detection	1	Positive
Kidney Beans	1	No detection	1	Positive
Soymilk	1	No detection	1	Positive
Chicken ^c	1	No detection	1	Positive
Shrimp	1	No detection	1	Positive
Octopus	1	No detection	1	Positive

^aAll samples were pre-screened before analysis utilizing Romer AgraStrip® Whole Egg and by ELISA utilizing Neogen Egg White Protein ELISA Kit.

^bN is the number of sample(s) tested.

^cPositive egg detection was determined utilizing Romer AgraStrip® Whole Egg and detection below the limit of quantification was determined when utilizing the Neogen Egg white Protein ELISA Kit.

Table 5. Matrix study evaluation at various concentrations of egg protein using the Egg White Protein Rapid Kit. (1)

Matrix (test portion) ^a	Target concentration of egg protein ^b (ppm)	N ^c	X ^d	POD ^e	95%CI ^f
Vanilla Ice Cream (0.2 g)	0	30	0	0.00	0.00, 0.11
	0.3	30	4	0.13	0.05, 0.30
	0.5	30	8	0.27	0.14, 0.44
	1	30	30	1.00	0.89, 1.00
	2	30	30	1.00	0.89, 1.00
Liquid Chocolate Syrup (0.5 mL)	0	30	0	0.00	0.00, 0.11
	0.3	30	5	0.17	0.07, 0.34
	0.5	30	12	0.40	0.25, 0.58
	1	30	28	0.93	0.79, 0.98
	2	30	29	0.97	0.83, 1.00
Fluid Whole Milk (0.1 mL)	0	30	0	0.00	0.00, 0.11
	0.3	30	4	0.13	0.05, 0.30
	0.5	30	11	0.37	0.22, 0.54
	1	30	29	0.97	0.83, 1.00
	2	30	30	1.00	0.89, 1.00
Orange Juice (0.1 mL)	1,200	10 ^g	N/A ^f	N/A	N/A
	0	30	0	0.00	0.00, 0.11
	0.3	30	5	0.17	0.07, 0.34
	0.5	30	18	0.60	0.42, 0.75
	1	30	30	1.00	0.89, 1.00
Salad Dressing (0.2 g)	2	30	30	1.00	0.89, 1.00
	0	30	0	0.00	0.00, 0.11
	0.3	30	4	0.13	0.05, 0.30
	0.5	30	10	0.33	0.19, 0.51
	1	30	30	1.00	0.89, 1.00
Incurred cookies (0.2 g)	2	30	30	1.00	0.89, 1.00
	0	30	0	0.00	0.00, 0.11
	0.5	30	14	0.47	0.30, 0.64
	1	30	30	1.00	0.89, 1.00
	2	30	30	1.00	0.89, 1.00
CIP Final rinse Water (0.2 mL)	0	30	0	0.00	0.00, 0.11
	0.3	30	4	0.13	0.05, 0.30
	0.5	30	13	0.43	0.27, 0.61
	1	30	30	1.00	0.89, 1.00
	2	30	30	1.00	0.89, 1.00
Stainless Steel ^h (10 x 10 cm)	0	5	0	0.00	0.00, 0.43
	0.0625	30	9	0.30	0.17, 0.48
	0.125	30	15	0.50	0.33, 0.67
	0.25	5	5	1.00	0.57, 1.00
	0.5	5	5	1.00	0.57, 1.00

^aAll matrixes were pre-screened to verify the absence of egg proteins utilizing both Romer AgraStrip® Whole Egg and 3M Egg White Protein ELISA Kit.

^bSpiked levels were confirmed utilizing Neogen Egg White Protein ELISA Kit.

^cN = Number of test portions.

^dx = Number of positive test portions.

^ePOD_c = Candidate method positive outcomes divided by the total number of trials.

^f95% CI = 95% Confidence Interval.

^gContamination level tested to determine hook effect; POD, CI, and Contamination level confirmation not needed per protocol. All samples tested at 1:1 dilution to obtain positive results.

^hUnits for the target concentration of egg protein for Stainless Steel are in µg of egg protein/100 cm².

REFERENCES CITED

- Schumacher, A., and Velasco, G.L., Validation of the 3M® Egg White Protein Rapid Kit for the Detection of Egg white Proteins in Select Foods and Environmental Surfaces, AOAC *Performance Tested Methods*SM certification number 052001.
- Schumacher, A., AOAC *Performance Tested Method*SM 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.