



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

052001

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

3MTM Egg White 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[®] *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 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".

Scott Coates, Senior Director
Signature for AOAC Research Institute

December 08, 2020

Date

METHOD AUTHORS April Schumacher and Gabriela Lopez Velasco	SUBMITTING COMPANY 3M Food Safety Department 3M Center, Bldg 275-5W-05 St. Paul, MN 55144
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KIT NAME(S) 3M™ Egg White Protein Rapid Kit	CATALOG NUMBERS L25EGG
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INDEPENDENT LABORATORY Q.Laboratories Cincinnati, OH 45204 USA	AOAC EXPERTS AND PEER REVIEWERS Yumin Chen ¹ , Joe Boison ² , John Szpylka ³ ¹ PepsiCO R&D, Barrington, IL, USA ² Consultant, Saskatoon, Canada ³ Food Safety Net Services, San Antonio, TX, USA
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APPLICABILITY OF METHOD
Target analyte – Egg White Protein

Matrixes – (100 µL): vanilla ice cream, whole fluid milk, orange juice, salad dressing
(500 µL) Liquid chocolate syrup
(0.2 g) incurred cookie
(200 µL) clean-in-place (CIP) final rinse water
environmental swab on stainless steel (10 x 10 cm surface area)

Performance claims - The sensitivity of the 3M™ 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 annually through December 2021
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METHOD MODIFICATION RECORD 1. December 2020 Level 1	SUMMARY OF MODIFICATION 1. Addition of 2 general warning statements regarding allergens.
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Under this AOAC® Performance TestedSM License Number, 052001 this method is distributed by: NONE	Under this AOAC® Performance TestedSM License Number, 052001 this method is distributed as: NONE
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PRINCIPLE OF THE METHOD (1)

The 3M™ 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 3M 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 3M 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 3M 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 3M Egg White Protein ELISA Kit and Romer AgraStrip® Whole Egg lateral flow assay. Results were negative for all matrixes except chicken; the 3M 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 3M 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 3M 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 3M Egg white Protein ELISA Kit.

Table 5. Matrix study evaluation at various concentrations of egg protein using the 3M Egg White Protein Rapid Kit. (1)

Matrix (test portion) ^a	Target concentration of egg protein ^b (ppm)	N ^c	X ^d	POD _c ^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
	1,200	10 ^g	N/A ^f	N/A	N/A
Orange Juice (0.1 mL)	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
CIP Final rinse Water (0.2 mL)	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	13	0.43	0.27, 0.61
	1	30	30	1.00	0.89, 1.00
Stainless Steel ^g (10 x 10 cm)	2	30	30	1.00	0.89, 1.00
	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 3M 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 TestedSM certification number 052001.