



# CERTIFICATION

## AOAC Research Institute *Performance Tested Methods*<sup>SM</sup>

Certificate No.  
**030403**

The AOAC Research Institute hereby certifies the method known as:

### **Veratox<sup>®</sup> for Peanut Allergen Test**

manufactured by  
**Neogen Corporation**  
620 Leshner Place  
Lansing, Michigan 48912  
USA

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> Program and found to perform as stated in the applicability of the method. 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*<sup>SM</sup> 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 that reads "Scott Coates".

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

Issue Date	December 26, 2023
Expiration Date	December 31, 2024

<b>AUTHORS</b> ORIGINAL VALIDATION: Douglas L. Park, Scott Coates, Vickery A Brewer, Eric A. E. Garber, Mohamed Abouzied, Kurt Johnson, Bruce Ritter, and Deborah McKenzie MODIFICATION AUGUST 2022: Marc Fowley, R. Lucas Gray, Brooke Roman, Robert Donofrio		<b>SUBMITTING COMPANY</b> Neogen Corporation 620 Leshar Place Lansing, Michigan 48912 USA
<b>METHOD NAME</b> Veratox® for Peanut Allergen Test		<b>CATALOG NUMBER</b> 8430
<b>INDEPENDENT LABORATORIES</b>	<b>Lab 3</b> National Food Processors Association 1350 I ST, NW Suite 300 Washington, DC	<b>APPLICABILITY OF METHOD</b> Target analyte – Peanut soluble proteins.  Matrixes – (5 g) - ice cream, breakfast cereal, cookies, milk chocolate
<b>Lab 1</b> FDA NE Regional Laboratory 158-15 Liberty Avenue Jamaica, NY USA	<b>Lab 2</b> Allergen Method Development Group, Food Research Division BCS Health Canada Sir Frederick Banting Research Center Ross Avenue, Building 22, PL 2203D Ottawa, Ontario, Canada	<b>Performance claims – The Veratox® for Peanut Allergen assay correctly identified in three laboratories 100% of sixty samples of breakfast cereal; cookies; ice cream; and milk chocolate contaminated with 5 ppm of peanut and correctly identified in three laboratories &gt;99% (239/240) of sixty samples of breakfast cereal; cookies; ice cream; and milk chocolate that did not contain any measurable amounts of peanut.</b>
<b>ORIGINAL CERTIFICATION DATE</b> March 13, 2003	<b>CERTIFICATION RENEWAL RECORD</b> Renewed annually through December 2024.	
<b>METHOD MODIFICATION RECORD</b> 1. August 2023 Level 2	<b>SUMMARY OF MODIFICATION</b> 1. Change in substrate composition for regulation and labelling requirements.	
<b>Under this AOAC Performance Tested Methods<sup>SM</sup> License Number, 030403 this method is distributed by:</b> NONE	<b>Under this AOAC Performance Tested Methods<sup>SM</sup> License Number, 030403 this method is distributed as:</b> NONE	

#### PRINCIPLE OF THE METHOD (1)

The Veratox for Peanut Allergen test is a sandwich enzyme-linked immunosorbent assay (S-ELISA). Peanut protein residue is extracted from samples with a phosphate buffered salt solution (PBS) by shaking in a heated water bath, followed by centrifugation or filtration. Extracted peanut residue is sampled and added to antibody-coated wells (capture antibody) where it binds to the antibody during an incubation. Any unbound peanut residue is washed away and a second antibody (detector antibody), which is enzyme labeled, is added. The detector antibody binds to the already bound peanut residue. After a second wash, substrate is added. Color develops because of the presence of bound detector antibody. Red Stop reagent is added and the color of the resulting solution is observed. The test is read in a microwell reader to yield optical densities. The optical densities of the controls form a standard curve, and the sample optical densities are plotted against the curve to calculate the exact concentration of peanut protein.

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

The 3 collaborative laboratories using the Veratox for Peanut Allergen Assay correctly identified 100% (240/240) of breakfast cereal, cookies, ice cream, and milk chocolate that were spiked with 5 ppm peanut and correctly identified >99% (239/240) of the samples of breakfast cereal, cookies, ice cream, and milk chocolate that did not contain any measurable amounts of peanut.

Table 1. Cross-reactivity with extracts <sup>a</sup> (1)	
Extract concentration	Veratox assay 40 mg/mL
Grains	
Barley	Negative
Buckwheat	Negative
Wheat	Negative
Wheat gluten	Negative
Rice	Negative
	Negative
Rye	Negative
Oat	Negative
Corn	Negative
Legumes and vegetables	
Soybean (desoy soya flour)	Negative
Green pea	Negative
Lima (butter) bean	Negative
Chick pea	Negative
Seeds	
Sunflower	Negative
Pumpkin	Negative
Sesame	Negative
Poppy	Negative
Nuts	
Almond	Negative
Brazil	Negative
Cashew	Negative
Chestnut	Negative
Coconut	Negative
Hazelnut	Negative
Macadamia	Negative
Pistachio	Negative
Pecan	Negative
Pine nut kernel	Negative
Walnut	Negative
Miscellaneous	
Skim milk powder	Negative
Cocoa	Negative
Lecithin	Negative
Bovine gelatin	Negative
Porcine gelatin	Negative
<sup>a</sup> Extracts were analyzed using the Veratox® for Peanut Allergen Assay at 40 mg/g by Neogen (Lansing, MI)	

**Table 2: Qualitative detection of peanut in spiked samples<sup>a</sup>(1)**

Kit	0 $\mu$ g peanut/g <sup>b</sup>					5 $\mu$ g peanut/g <sup>b</sup>				
	Cereal	Cookies	Milk chocolate	Ice cream	Total	Cereal	Cookies	Milk chocolate	Ice cream	Total
Veratox® Assay	59/60	60/60	60/60	60/60	239/240	60/60	60/60	60/60	60/60	240/240

<sup>a</sup> All samples were analyzed by the participating laboratories within 7 days of receipt. Data were compiled by the AOAC Research Institute.

<sup>b</sup> The fraction of correctly identified samples for each of the commodities along with the total for each level spiked.

**DISCUSSION OF THE MODIFICATION APPROVED AUGUST 2022 (2)**

Based on the data collected in this study, the performance of each Veratox test kit met Neogen’s acceptance criteria for recovery and repeatability relative standard deviation with each test lot of substrate. The newly formulated substrate also performed well in real-time stability testing.

**Table 9. Veratox for Peanut Lot to Lot Substrate Comparison (2)**

Substrate	Level, mg/kg	N <sup>a</sup>	Mean, ppm	s <sub>r</sub> <sup>b</sup>	RSD <sub>r</sub> , % <sup>c</sup>	Rec., % <sup>d</sup>	Bias <sup>e</sup>
K-Blue	NDA	5	0.0	0.00	N/A	N/A	0.0
Reference lot	5	5	4.1	0.61	15.0	81.2	-0.9
(lot 210325-02)	15	5	18.9	0.95	5.0	125.9	3.9
K-Blue	NDA	5	0.0	0.00	N/A	N/A	0.0
Test lot 1	5	5	4.1	1.11	27.2	82.0	-0.9
(lot 210723-01)	15	5	15.9	3.40	21.8	105.7	0.9
K-Blue	NDA	5	0.4	0.42	N/A	N/A	0.0
Test lot 2	5	5	4.9	1.06	21.8	97.2	-0.1
(lot 210805-01)	15	5	18.0	3.24	18.0	120.3	3.0
K-Blue	NDA	5	0.0	0.00	N/A	N/A	0.0
Test lot 3	5	5	5.1	0.96	18.9	101.2	0.1
(lot 210304)	15	5	17.1	1.60	9.4	113.7	2.1

<sup>a</sup> N = Number of determinations (five replicate test portions, extraction through detection, three analyses of each portion).

<sup>b</sup> s<sub>r</sub> = Repeatability standard deviation.

<sup>c</sup> RSD<sub>r</sub> = Repeatability relative standard deviation.

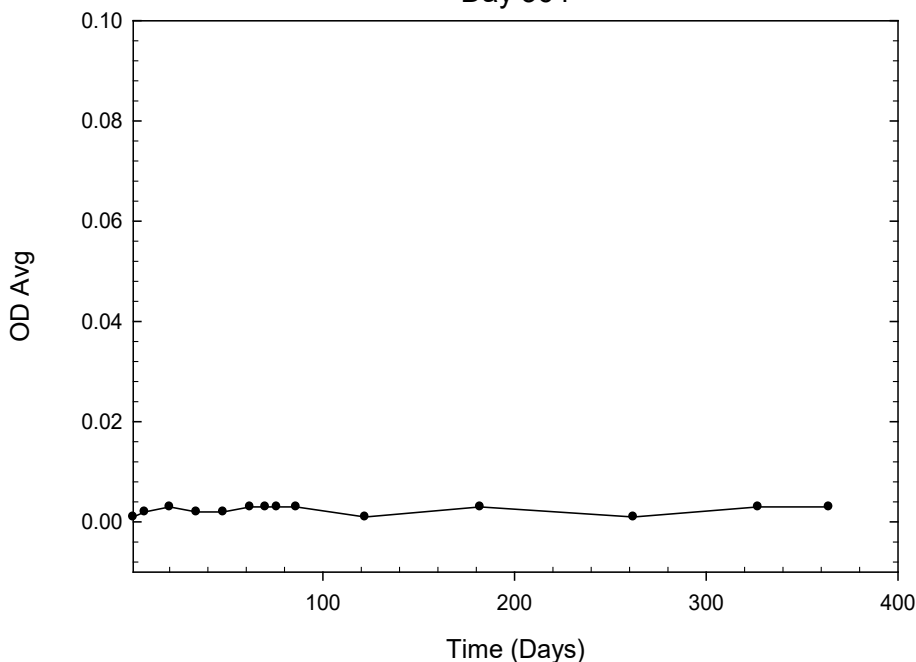
<sup>d</sup> Recovery = (mean<sub>cand</sub>/known concentration) x 100.

<sup>e</sup> Bias = mean<sub>cand</sub> - known concentration.

Veratox for Peanut ANOVA Results

No lots differed significantly (P<0.05)

K-Blue Advanced Plus TMB Substrate  
Background Stability at 4°C  
1 cm pathlength vs H<sub>2</sub>O Blank at 650 nM  
Day 364



**Figure 2: Background stability of substrate stored under ideal conditions and over a period of 364 days. (2)**

**REFERENCES CITED**

1. Park, D.L., Coates, S., Brewer, V.A., Garber, E.A.E., Abouzied M., Johnson, K., Ritter, B., and McKenzie, D, *Performance Tested Methods<sup>SM</sup> Multiple Laboratory Validation Study of ELISA-Based Assays for the Detection of Peanuts in Food, AOAC Performance Tested Methods<sup>SM</sup> certification number 030403.*
2. Fowley, M., Gray, R.L., Roman, B., and Donofrio, R., *Level 2 Modification Study to Validate Multiple Veratox® ELISA Methods, AOAC Performance Tested Methods<sup>SM</sup> certification number 030403. Approved August 3, 2022.*