

# **Certification Report**

**AOAC Research Institute**

**Performance *Tested Method***

**030403**

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

manufactured by

**Neogen Corporation  
620 Leshar Place  
Lansing, MI 48912 USA**



**May 13, 2003**

## **General Information**

The Neogen Corporation Veratox<sup>®</sup> for Peanut Allergen has been validated and certified as a *Performance Tested Method* by the AOAC Research Institute as an effective method for the detection of peanut proteins at levels as low as 5ppm in a variety of foods including breakfast cereal; cookies; ice cream; and milk chocolate.

The Veratox<sup>®</sup> for Peanut Allergen is a sandwich enzyme-linked immunosorbent assay (S-ELISA). Peanut protein is extracted from samples with a buffered salt solution (PBS) by shaking in a heated water bath, followed by filtration. Extracted peanut protein is sampled and added to antibody-coated wells (capture antibody) where it binds to the antibody during a 10 minutes incubation. Any unbound protein is washed away and a second antibody (detector antibody), which is enzyme-labeled is added. During second 10 minutes incubation, the detector antibody binds to the already bound peanut protein. After a second wash, the substrate is added. Color develops as a result of the presence of bound detector antibody. A stopping 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 from a standard curve and the sample optical densities are plotted against the curve to calculate the exact concentration of the peanut. The test also can be used for qualitative purpose by visually comparing the blue color intensity of the sample to blue color intensity of the desired control. If the sample well has more blue color than the control well, the sample contains more peanut protein than the control. If the sample well has less blue color or more red color, than the control well, the sample contains less peanut protein than the control.

- 1.0 Target analyte**– peanut.
- 2.0 Matrices** – including breakfast cereal; cookies; ice cream; and milk chocolate.
- 3.0 Summary of validated performance claims** – The Veratox<sup>®</sup> 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 >99% (239/240) of sixty samples of breakfast cereal; cookies; ice cream; and milk chocolate that did not contain any measurable amounts of peanut..

The Veratox<sup>®</sup> for Peanut Allergen assay was challenged with 32 potentially cross-reactive proteins including: barley, buck wheat, wheat, wheat gluten, rice, rye, oats, corn, soy bean, peas, lima beans, chick peas, sunflower seeds, pumpkin seeds, sesame, poppy seed, almond, brazil nut, cashew, chestnut, coconut, hazelnut, macadamia, pistachio, pecan nut, pine nut, walnut, milk proteins as skim milk powder, cocoa, lecithin, bovine gelatin, and porcine gelatin. The Veratox<sup>®</sup> for Peanut Allergen assay correctly identified as negative all 32 potentially cross-reactive proteins thereby demonstrating that the test kit method has no cross-reactivity and is very specific to peanut.

#### **4.0 Test Kit Information**

4.1 Kit name- Veratox<sup>®</sup> for Peanut Allergen

4.2 Catalog Number- 8430

4.3 Ordering Information

U.S.A.-Neogen Corporation, 620 Lesher Place, Lansing, MI 48912  
Phone: 517-372-9200  
Fax: 517-372-0108  
Website: [www.neogen.com](http://www.neogen.com)

Rest of the world- [www.neogen.com](http://www.neogen.com) for local distributor information.

#### **5.0 Test kit reagents:**

5.1 Antibody coated solid support - 12 well coated microwell strips. Store at 4°C.

5.2 Liquid enzyme conjugate – Horseradish peroxidase enzyme-labeled to polyclonal antibody specific peanut protein. Store at 4°C.

5.3 Mixing wells - 12 well uncoated microwell strips.

5.4 Enzyme substrate - K-Blue Substrate<sup>®</sup> (Tetramethylbenzidine) or equivalent.

5.5 Red Stop enzyme stopping solution - Contains 6.0 mg NaF, 400 mg Na<sub>4</sub>EDTA, 6 mL 1M

5.6 NaOH, 8.18g NaCl, 1.0 mL (0.5%) Q.S. 44 (detergent), red food coloring. Store at 4°C.

5.7 Peanut Controls – Liquid extract of peanut contains 0, 100, 200, 400, and 1000 ng/mL (corresponding to a level of peanut in the tested samples of 0, 2.5, 5, 10 and 25 ppm, respectively, considering the 1:25 dilution factor)

5.8 Foil pouch of 10 mM PBS dry powder extraction solvent. Each pouch is enough to prepare 1L in distilled water at pH 7.4.

5.9 Washing buffer-40 mL of concentrated PBS–Tween solution. Dilute with 960 mL distilled water to produce a washing reagent containing 10 mM PBS, pH 7.4 and 0.05% Tween- 20.

5.10 Extraction additive- Proprietary formula – available only from Neogen Corporation.

5.11 Measuring scoop.

## **6.0 Additional Supplies and Reagents**

6.1. Distilled or deionized water.

6.2 250 mL Erlenmeyer flask (one for each sample).

6.3 Filter paper - Whatman No. 4 or equivalent.

6.4 Pipet and pipet tips - 100 µL Medical Laboratories brand (MLA) or equivalent.

6.5 Microwell holder- Dynatech 96 well holder or equivalent.

6.6 Graduated cylinder - 250 mL capacity.

6.7 Reagent Boats (3) – Plastic trough with 30 mL capacity one each for conjugate, substrate and stopping reagent.

## **7.0 Apparatus**

7.1 Shaker water bath – capable of maintaining 60°C and 50 rpm.

7.2 Multichannel pipet - Labsystems 12-channel or equivalent

7.3 EIA Reader- Microwell spectrophotometer with 650 nm filter. Awareness Technologies Stat Fax 303 or equivalent.