Quantitative Egg Allergen Test

Questions? Call 800-234-5333 or 517-372-9200

11. PARAFILM® M laboratory film
12. 1 L bottle to prepare washing solution (Neogen item #9472)
13. 1 L heat safe bottle to prepare extract solution (Neogen item #9472)
14. Paper towels or equivalent absorbent material
15. Microwell holder (Neogen item #9402)
16. Waterproof marker
17. Wash bottle (Neogen item #9400)
18. Distilled or deionized water
19. 3 reagent boats for multichannel pipettor (Neogen item #9435)
20. Graduated cylinder capable of measuring 125 mL (Neogen item #9368)
21. Scale capable of weighing 5 g (Neogen item #9427)

**PRECAUTIONS**

1. Components of Veratox for Egg Allergen, such as controls and extraction reagents, may contain one or more of the following potentially allergic materials: casein; egg protein; peanut protein; soy protein; tree nut protein. If allergic to any of these compounds, please use caution when using this product.
2. Store test kit between 2-8°C (35-46°F) when not in use.
3. Do not use kit components beyond expiration date.
4. Do not mix reagents from one kit serial with reagents from a different kit serial.
5. Do not run more than 24 wells per test.
6. Follow proper pipetting techniques (e.g., prime tips, using clean tips).
7. Use only incubation times specified. Others may give inaccurate results.
8. Bring kits to room temperature (18-30°C, 64-86°F) prior to use.
9. Avoid prolonged storage of kits at ambient temperatures.
10. Filter the extract by pouring at least 5 mL through a Whatman #4 filter paper. Reagent boat to keep the substrate protected from direct light and contaminants.
11. Use clean glassware for each sample to avoid cross-contamination. Thoroughly wash all glassware between samples.

**PROCEDURAL NOTES**

1. **Substrate.** K-Blue Substrate is ready for use. The substrate should be clear to light blue — discard if it has turned dark blue. Only pour the needed volume of substrate into a reagent boat. **Do not return unusable substrate to the bottle.** Cover the reagent boat to keep the substrate protected from light until needed.
2. **Conjugate.** The conjugate supplied with this kit is ready to use. One bottle is enough for 24 wells. Cover the reagent boat to keep the conjugate protected from direct light and contaminants.
3. **Extraction solution.** Prepare extraction solution by adding a foil pouch of extraction solvent, 10mM PBS, to 1 L distilled or deionized water. Swirl to mix thoroughly. Cover and store any unused portions refrigerated at 2-8°C (35-46°F).
4. **Wash buffer.** Prepare the wash buffer solution by pouring all the wash buffer concentrate into an empty 1 L container. Rinse the wash buffer concentrate bottle with distilled or deionized water and pour into the 1 L container to ensure all the concentrate is used. Fill the 1 L container with additional distilled or deionized water, and swirl to assure thorough mixing. Cover and store any unused portions refrigerated at 2-8°C (35-46°F).

**TEST PROCEDURE**

Allow the test kit and all reagents to warm to room temperature (18-30°C, 64-86°F) before using.

1. Remove 1 red-marked mixing well for each sample to be tested plus 5 red-marked wells for controls, and place in the well holder.
2. Remove an equal number of antibody-coated wells. Return antibody wells which will not be used immediately to the foil pack with desiccant. Reeat the foil pack to protect the antibody. Mark one end of the strip with a “1”, and place strip in the well holder with the marked end on the left.
3. Mix each reagent by swirling the reagent bottle prior to use.
4. Using a new pipette tip for each, transfer 150 µL of controls and sample extracts to the red-marked transfer wells as shown in the template below. Only run up to two 12-well strips at a time. Place tips on the 12-channel pipettor and transfer 100 µL of the controls and sample extracts to the antibody-coated wells. Mix for 20 seconds by sliding the well holder back and forth on a flat surface.
5. Incubate microwells 10 minutes at room temperature (18-30°C, 64-86°F). Discard the transfer control wells.
6. Empty the contents of the wells into a sink. With a wash bottle fill each antibody well with the wash buffer solution and dump out. Repeat the washing 5 times, then turn the wells upside down and tap out on a paper towel until the remaining washing solution is removed.
7. Pour the needed volume of conjugate from the blue-labeled bottle into a clean reagent boat.
8. Using the 12-channel pipettor and new tips, transfer 100 µL of the conjugate into all the wells and mix for 20 seconds by sliding the well holder back and forth on a flat surface.
9. Incubate for 10 minutes at room temperature (18-30°C, 64-86°F).
10. Wash all wells with the wash buffer solution as described in step 7. Incubate for 10 minutes at room temperature (18-30°C, 64-86°F).
11. Discard the needed volume of substrate solution from the green-labeled bottle into a clean reagent boat.
12. Place new tips on the 12-channel pipettor and transfer 100 µL of substrate into each well and mix for 20 seconds. Do not eject tips.
13. Incubate for 10 minutes at room temperature (18-30°C, 64-86°F).
14. Mix each reagent by swirling the reagent bottle prior to use.

**PERFORMANCE CHARACTERISTICS**

**Limit of quantitation:** 2.5 ppm (Described as the lowest concentration of Red Stop solution to the red-marked transfer wells as shown in the template below. Only run up to two 12-well strips at a time. 0 2.5 5 10 25 50 100 200 ppm)

**Range of quantitation:** 2.5 – 25 ppm (For quantitating samples above 25 ppm, contact a Neogen representative for dilution instructions.)

**NOTE:** Due to variations in food additives and commodity compositions, levels below 10 ppm may be considered suitable for research purposes only.
Egg Allergy
Food allergens are proteins in food that can create an immune response in sensitive individuals. Once ingested, food allergens can cause a number of reactions, ranging in severity from hives and itching to anaphylaxis. Anaphylaxis is a severe allergic reaction, involving vomiting, diarrhea, difficulty breathing, swelling of the mouth and tongue, and a rapid drop in blood pressure. An estimated 2 to 3 percent of adults, and 5 to 8 percent of children, are sensitive in some degree to food allergens. More than 6 million people in the United States alone are known to have a food allergy, with an allergy to eggs being one of the most prevalent.

Food manufacturers protect those with food allergies by clearly labeling their products with a list of ingredients. Food manufacturers also protect those with a food allergy by clearly labeling their products with a list of ingredients. Testing for the presence of egg components ensures food manufacturers that an unlabeled—and potentially dangerous—ingredient did not make its way into a food product.

INTENDED USE
Veratox for Egg Allergen is intended for the quantitative analysis of unprocessed and heat processed egg protein in food products such as pasta, salad dressing, cake mix and ice cream.

INTENDED USER
This test kit is designed for use by quality control personnel and others familiar with foods possibly contaminated by eggs or egg products. Since technique is very important, operators should be trained by a Neogen representative or someone who has completed the Neogen training.

ASSAY PRINCIPLES
Veratox for Egg Allergen test is a sandwich enzyme-linked immunosorbent assay (S-ELISA). Egg protein is extracted from samples with a buffered salt solution (PBS) by shaking in a heated water bath, followed by centrifugation or filtration. Extracted egg protein is sampled and added to antibody-coated wells (capture antibody) where it binds to the antibody during an incubation. Any unbound protein is washed away and a second antibody (detector antibody), which is enzyme labeled, is added. The detector antibody binds to the already bound egg protein. After a second wash, the substrate is added. Color develops as a result 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 allergen.

STORAGE REQUIREMENTS
The kit can be used until the expiration date on the label when stored refrigerated at 2-8°C (35-46°F).

MATERIALS PROVIDED
1. 48 antibody-coated microwells
2. 48 red-marked transfer wells
3. 5 yellow-labeled bottles of 1.5 mL each 0.2, 5, 10, 25 ppm egg protein control
4. 2 blue-labeled bottles of 5.0 mL each enzyme-labeled antibody conjugate
5. 1 green-labeled bottle of 24 mL K-Blue® Substrate
6. 1 red-labeled bottle or 32 mL Red Stop solution
7. Foil pouch of 10 mL PBS dry powder extraction solvent. Each pouch is enough to prepare 1 L in distilled or deionized water (pH 7.4)
8. 40 mL of 10 mM PBS-Tween washing reagent in a wide mouth bottle. Each bottle is enough to prepare 1 L in distilled or deionized water
9. 50 grams of extraction additive in a specimen cup
10. Plastic scoop to measure extraction additive
11. Directions for use

MATERIALS RECOMMENDED BUT NOT PROVIDED
1. Allergen Extraction Kit (Neogen item #8429)
   a. 20 disposable plastic extraction bottles
   b. 20 sample collection tubes (12x75 mm) with caps
2. 250 mL Erlenmeyer flasks (one per sample, if not using extraction kit)
3. Shaker bath water capable of maintaining 60°C ± 1° with clamps to hold 250 mL Erlenmeyer flasks
4. Whatman #4 filters or equivalent
5. Centrifuge (optional)
6. 50-200 µL adjustable pipettor (Neogen item #9276)
7. 12-channel pipettor (Neogen item #9273)
8. Pipette tips (Neogen item #9410)
9. Timer (Neogen item #9426)
10. Microwell reader with a 650 nm filter (Neogen item #9301/9302)