PROCEDURAL NOTES

1. Components of Veratox for Lupine Allergen Kit, such as controls and extraction reagents, may contain one or more of the following potentially allergic materials: lupine protein; casein; egg protein; soy protein; tree nut protein. If allergic to any of these compounds, please use caution when using this product.

2. Concentrated food additives, colors and flavors may cause interferences on ELISA test methods. Contact Neogen’s technical services for information if needed.

3. Hydrolyzed and fermented proteins may not be detected using ELISA methods for allergen testing. Due to the breakdown of the proteins to small peptides or amino acids, they may become undetectable by this assay, but still could cause an allergic and cause an allergic reaction.

4. Store test kit between 2-8°C (35-46°F) when not in use, do not freeze.

5. Bring kits to room temperature (18-30°C, 64-86°F) prior to use.

6. Avoid prolonged storage of kits at ambient temperatures.

7. Do not use kit components beyond expiration date.

8. Do not mix reagents from one kit serial with reagents from a different kit serial.

9. Do not run more than 24 wells per test.

10. Follow proper pipetting techniques (e.g., prime tips and use clean tips).

11. Use only incubation times specified. Others may give inaccurate results.

12. Use clean pipette tips and 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 unused 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 buffer, 10 mM PBS, to 1 L distilled or deionized water. Mix by swirling thoroughly dissolve buffer. 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).

NOTE: Discard unused portions of extraction solution and wash buffer when the test kit has been used completely.

5. Antibody wells. Keep wells sealed in the foil pouch until needed. Remove wells from the foil pouch only after samples are extracted, and the test procedure is set to begin.

SAMPLE PREPARATION AND EXTRACTION

The sample to be tested should be collected according to accepted sampling techniques (see Neogen’s Food Allergen Handbook). The sample should be ground and thoroughly mixed prior to proceeding with the extraction procedure.

1. Prepare the extraction solution as described in the procedural notes.

2. Incubate for 10 minutes at room temperature (18-30°C, 64-86°F). Discard the red-marked transfer wells.

8. Pipet a 100 µL volume of conjugate from the blue-labeled bottle into a clean reagent boat.

9. Use 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.

10. Incubate for 10 minutes at room temperature (18-30°C, 64-86°F).

11. Wash all wells with the wash buffer solution as described in step 7.

12. Pour the needed volume of substrate solution from the green-labeled bottle into a clean reagent boat.

13. 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.


15. Pour the needed volume of Red Stop solution from the red-labeled bottle into a clean reagent boat.

16. With the same tips used to dispense the substrate, transfer 100 µL of Red Stop into each well and mix for 20 seconds.

17. Wipe the bottom of the microwells and read in a microwell reader with a 650 nm filter.

18. Interpret the test results using Neogen’s StatFax microwell reader, or an equivalent strip reader. If using a strip reader, calculate the results using Neogen’s Log/Logit software.

PERFORMANCE CHARACTERISTICS

Limit of quantitation: 2.5 ppm (Described as the lowest concentration point on the calibration curve that this test can reliably detect lupine protein.)

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

Allergen detection: This test detects lupine proteins and the results are expressed as ppm of lupine.

NOTE: Due to variations in food additives and commodity compositions, levels below 10 ppm may be considered suitable for research purposes only.
LUPINE ALLERGEN

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 3.5 to 4 percent of adults, and 6 to 8 percent of children, are sensitive in some degree to food allergens. More than 12 million people in the United States alone are known to have a food allergy. Food manufacturers protect those with food allergies by clearly labeling their products with a list of ingredients. Testing for the presence of lupine proteins ensures food manufacturers that an unlabeled—and potentially dangerous—ingredient did not make its way into a food product.

INTENDED USE

Veratox for Lupine Allergen is intended for the quantitative analysis of lupine proteins in food products such as flours, cake mixes and clean-in-place rinses.

INTENDED USER

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

ASSAY PRINCIPLES

The Veratox Lupine Allergen Test is a Sandwich Enzyme-Linked Immunosorbent Assay (5-ELISA). Lupine protein residue is extracted from samples with a buffered salt solution (PBS) by shaking in a heated water bath, followed by centrifugation or filtration. Extracted lupine protein is sampled and added to antibody-coated wells (capture antibody) where it binds to the antibody during an incubation. Any unbound lupine protein is washed away and a second antibody (detector antibody), which is enzyme labeled, is added. The detector antibody binds to the already bound lupine protein. After a second wash, substrate is added. Color develops as a result of the presence of bound detector antibody. Red Stop reagent is added and the color 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 lupine, expressed as ppm lupine.

STORAGE REQUIREMENTS

The kit is 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 0, 2.5, 5, 10, 25 ppm lupine controls
4. 2 blue-labeled bottles of enzyme-labeled antibody conjugate
5. 1 green-labeled bottle of K-Blue® Substrate
6. 1 red-labeled bottle of Red Stop solution
7. 5 pouches of 10 mM PBS dry powder extraction buffer. Each pouch is enough to prepare 1 L in distilled or deionized water.
8. 40 mL of 25x 10 mM PBS-Tween washing reagent in a wide mouth bottle. Each bottle is enough to prepare 1 L in distilled or deionized water (pH 7.4)
9. 50 grams of extraction additive in a specimen cup
10. Plastic scoop to measure extraction additive

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. Shaker water bath capable of maintaining 60°C ± 1°C with clamps to hold 250 mL extraction bottles
3. Whatman #4 filters or equivalent
4. Centrifuge (optional)
5. Filter funnel, 65 mm (Neogen item #9353)
6. Pipettor, 50-200 µL, adjustable (Neogen item #9276)
7. Pipettor, 12-channel (Neogen item #9273)
8. Pipette tips (Neogen item #84109407)
9. Timer (Neogen item #9426)
10. Microwell reader with a 650 nm filter (Neogen item #9302)
11. Bottle, 1 L, to prepare washing solution (Neogen item #9472)
12. Bottle, 1 L, heat safe, to prepare extraction solution (Neogen item #9472)
13. Paper towels or equivalent absorbent material
14. Microwell holder (Neogen item #9402)