U.S. English

Product Number:
8570

Veratox®

VIP for Cashew

Refrigerate at 2–8°C (35–46°F). Do not freeze

NEOGEN®

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Cashew Allergen

Food allergens are proteins in food that can create an immune response in sensitive individuals. Adverse reactions vary and range 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. More than 32 million people (9%) of the population in the U.S. alone are known to have a food allergy, six million of which are under the age of 18. Food manufacturers protect those with food allergies by clearly labeling their products with a list of ingredients. Testing for the presence of cashew components ensures food manufacturers that an unlabeled — and potentially dangerous — ingredient did not make its way into a food product.

Intended Use

Veratox® VIP for Cashew is intended for the quantitative analysis of cashew protein residue in food products, including ultra-high temperature processed beverages, food ingredients, and clean-in-place (CIP) rinses.

Intended User

This test kit is designed for use by quality control personnel and others familiar with foods possibly contaminated by cashews or cashew 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 VIP for Cashew is a sandwich enzyme-linked immunosorbent assay (S-ELISA). Cashew residues are extracted from samples with a buffered salt solution by shaking in a heated water bath, followed by centrifugation or filtration. Extracted cashew residue is sampled and added to capture antibody-coated wells where it binds to the antibody during an incubation. Any unbound cashew residue is washed away, and a second enzyme-labeled detector antibody is added. The detector antibody binds to the already bound cashew residue. After a second wash, the substrate is added. Color develops as a result of the presence of bound detector antibodies. Red Stop Solution 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 cashew residue.

Storage Requirements

The kit can be used until the expiration date on the label when stored refrigerated at 2–8°C (35–46°F). Do not freeze or expose to temperatures exceeding 37°C for prolonged periods.

Materials Provided

1. 48 antibody-coated microwells
2. 48 red-marked transfer wells
3. 6 yellow-labeled bottles of 0, 0.2, 0.5, 1, 2, and 5 ppm cashew protein 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. One bottle of 25x allergen extraction buffer (AEB). Each bottle is enough to prepare 5 L of extraction solution 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 (pH 7.4)
9. 200 g of extraction additive in a specimen cup
10. One foil pouch of 10 mM PBS dry powder dilution solvent (for use with diluting high positive samples), enough to prepare 1 L in distilled or deionized water (pH 7.4)

Materials Recommended but Not Provided

1. Allergen extraction kit (optional)
   a. 20 disposable plastic extraction bottles
   b. 20 sample collection tubes (12 x 75 mm) with caps
2. Allergen environmental swabbing kit (Neogen item 8432S)
   a. 100 sterile swabs
   b. 100 dropper tips
3. Shaker water bath capable of maintaining 60 ± 1°C with clamps to hold 250 mL disposable plastic bottles
4. Whatman #4 filters or equivalent (Neogen item 9429)
   or
   Centrifuge (Neogen item number 9330)
5. Pipettor, adjustable 50–200 µL (Neogen item 9276)
6. Pipettor, 12-channel (Neogen item 9273)
7. Pipette tips (Neogen item 9410, 9407, 9417)
8. Timer (Neogen item 9426)
9. Microwell reader with a 650 nm filter (Neogen item 9303)
10. 1 L bottle to prepare washing solution (Neogen item 9472)
11. 1 L heat-safe bottle to prepare extract solution (Neogen item 9472)
12. Paper towels or equivalent absorbent material
13. Microwell holder (Neogen item 9402)
14. Waterproof marker
15. Wash bottle (Neogen item 9400)
16. Distilled or deionized water
17. 3 reagent boats for 12-channel pipettor (Neogen item 9435)
18 Graduated cylinder capable of measuring 125 mL (Neogen item 9368)
19. Scale capable of weighing 5 ± 0.1 g (Neogen item 9427)

Precautions

1. Components of Veratox VIP for Cashew, such as controls and extraction reagents, may contain one or more of the following potentially allergic materials: casein, whey protein, and cashew 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 with ELISA test methods. Contact Neogen Technical Services for validation information.
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 be allergenic and cause an allergic reaction.
4. Sponges should not be used for sample collection and allergen testing. Sample collection swabs other than Neogen swabs should be validated prior to use. General sponges and swabs may contain solutions or materials that may interfere with the test kit.
5. Store test kits between 2–8°C (35–46°F) when not in use. Do not freeze test kits, and avoid prolonged storage of kits at ambient temperatures.
6. Bring kits to room temperature 18–30°C (64–86°F) prior to use.
7. Cool sample extracts to room temperature before testing.
8. Do not use kit components beyond the expiration date.
9. Do not mix reagents from one kit serial with reagents from a different kit serial.
10. Do not run more than 24 wells per test when running the quantitative method.
11. When running the screening procedure, do not run more than 6 wells unless using a multichannel pipettor.
12. Follow proper pipetting techniques (e.g., prime tips and use clean tips).
13. Use only incubation times specified. Others may give inaccurate results.
14. 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 the 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. 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.
4. Extraction solution: Prepare extraction solution by adding one part of the 25x AEB into 24 parts of distilled/deionized water (pH 7.4) (e.g., to make 1 L of extraction solution, add 40 mL of 25x AEB into 960 mL of water). Swirl to mix thoroughly. Cover and store any unused portions refrigerated at 2–8°C (35–46°F).
5. Washing solution: Prepare the washing 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).
6. Dilution solution: If you have samples that have levels of cashew protein above the quantitation curve, these samples must be diluted in prepared dilution solution and reanalyzed. To prepare dilution solution, add foil pouch of 10 mM PBS to 1 L distilled or deionized water. Swirl to mix thoroughly. Cover and store any unused portions at 2–8°C (35–45°F). **Note**: Discard unused portions of extraction solution and wash buffer when the test kit has been used completely.

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. For collecting and extracting environmental swabs, refer to the Allergen Environmental Swabbing Kit (Neogen item 8432S) instructions.

1. Prepare the extraction solution as described in the procedural notes.
2. Preheat the extraction solution to 60°C (140°F) by immersing the bottle containing the solution into the water bath and allowing it to reach 60°C.
3. Using your sampling and collection procedure, obtain a representative sample and grind it to a very fine particle size.
4. Transfer 5 g of sample or 5 mL of liquid sample into a disposable extraction bottle.
5. Add 5 g of extraction additive to the sample bottle. Do not use the extraction additive from another allergen test kit.
6. Pour 100 mL of the 60°C (140°F) extraction solution into the sample bottle.
7. Cap the sample bottle to prevent contents from splashing during the extraction. Mix or vortex until all components are in solution.
8. Extract by shaking (150 rpm) in a water bath at 60°C (140°F) for 15 minutes. Remove the bottle from the bath.
9. Let material settle for 5 minutes to enable some of the sample to settle before proceeding to the next step.
10. Centrifuge at 14,000 RPM for 5 minutes (20 min for lower speeds), using the clear supernatant as the sample or filter the extract by pouring at least 5 mL through a Whatman #4 filter and collecting the filtrate as the sample.
    Note: Internal data have shown centrifugation demonstrates the most consistent recovery.
11. Allow extracts to cool to room temperature before beginning analysis.
12. Discard extracts after completion of analysis.

Test Procedure for Quantitation

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 6 red-marked wells for controls, and place in the well holder.
2. Remove an equal number of antibody-coated wells. Return antibody wells that will not be used immediately to the foil pack with desiccant. Reseal the foil pack to protect the antibody. Mark one end of the strip with a 1, and place the 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 2, 12-well strips at a time.

0	0.2	0.5	1	2	5	S1	S2	S3	S4	S5	S6
S7	S8	S9	S10	S12	S13	S14	S14	S15	S16	S17	S18

5. 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.
6. Incubate microwells 10 minutes at room temperature 18–30°C (64–86°F). Discard the red-marked transfer wells.
7. Empty the contents of the wells into a sink. With a wash bottle, fill each antibody well with the washing solution and dump it out. Repeat the washing 5 times, then turn the wells upside down and tap out on a paper towel until all washing solution is removed.
8. Pour the needed volume of the conjugate from the blue-labeled bottle into a clean reagent boat.
9. 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.
10. Incubate for 10 minutes at room temperature 18–30°C (64–86°F).
11. Wash all wells with the washing 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 the substrate into each well and mix for 20 seconds. Do not eject tips.
14. Incubate for 10 minutes at room temperature 18–30°C (64–86°F).
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 Solution 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. Air bubbles should be eliminated, as they could affect analytical results. Results should be read within 20 minutes after the addition of Red Stop Solution.
18. Interpret the test's results using Neogen's microwell reader or an equivalent strip reader. If using a strip reader, calculate the results using Neogen's Veratox® for Windows software.
19. If the sample contains cashew protein levels higher than the highest standard (5 ppm cashew protein), sample extracts should be further diluted in PBS solution (preparation instructions included in procedural notes) and reanalyzed to obtain accurate results. Dilution factor must be accounted for when calculating results.

Test Procedure for Screening

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

1. Remove 1 well for each sample to be tested plus 1 well for the control, and place it into the well holder.
2. Choose the 0.5 ppm yellow-labeled control bottle to serve as the screening level for the test.
3. Mix each reagent by swirling its bottle before use.
4. Add 100 µL from the yellow-labeled control bottle to the first well. Next, add 100 µL from each sample extract to a respective well as indicated in the template below. For environmental swabs, add 3 drops from the swab tube with a dropper tip. Mix for 20 seconds by sliding the microwell holder back and forth on a flat surface.
   Control S1 S2 S3 S4 S5
5. Incubate microwells 10 minutes at room temperature 18–30°C (64–86°F).
6. Shake out the contents of the wells. Using a wash bottle filled with wash buffer solution, fill each well and shake out. Repeat 5 times. Remove excess wash buffer by turning wells upside down and vigorously tapping wells on an absorbent towel.
7. Add 100 µL from the blue-labeled conjugate bottle to each well. Mix for 20 seconds by sliding the microwell holder back and forth on a flat surface.
8. Incubate for 10 minutes at room temperature 18–30°C (64–86°F).
9. Shake out the contents of the wells. Using a wash bottle filled with washing solution, fill each well and shake out. Repeat 10 times. Remove excess washing solution by turning wells upside down and vigorously tapping wells on an absorbent towel.
10. Add 100 µL from the green-labeled substrate bottle to each well. Mix for 20 seconds by sliding the microwell holder back and forth on a flat surface.
11. Incubate for 10 minutes at room temperature 18–30°C (64–86°F).
12. Add 100 µL from the red-labeled Red Stop Solution bottle to each well. Mix for 20 seconds by sliding the microwell holder back and forth on a flat surface. The results are now ready to be interpreted.
13. Visually compare the color of a sample well to the color of the control well. If the sample well has more blue color than the control well, the sample tests positive for cashew contamination of more than the control used. If the sample well has a less blue color or more red color than the control well, the sample contains less than the control used of cashew contamination.
    Alternative: Read wells (wipe the bottom of wells with a dry cloth or towel first) in a microwell reader with a 650 nm filter. If the sample well has an optical density (OD) higher than the control well, the sample is positive for cashew contamination of more than the control used. If the sample well has an OD lower than the control well, the sample contains less than the control used of cashew contamination.

Performance Characteristics

Limit of quantitation: 0.2 ppm cashew protein (see appendix B). The limit of quantitation is described as the lowest concentration point on the calibration curve that this test can reliably detect cashew.

Range of quantitation: 0.2–5 ppm cashew protein (see appendix B). For quantifying samples above 5 ppm, contact a Neogen representative for dilution instructions.

Allergen detection: This test detects cashew protein, and the results are expressed as ppm of cashew protein.

Cross-reactivity: None shown.

Appendix A: Unit Conversion

This test report results in parts per million (ppm). This is equivalent to reporting in milligrams/kilogram (mg/kg).

Appendix B: Protein Conversion

Description	Result
Total Cashew	1–25 ppm
Cashew Protein	0.2–5 ppm
Conversion Factor*	20% Protein in Cashew

*USDA National Nutrient Database (cashews, raw; cashews, dry roasted; cashews, oil roasted)

Customer Service

Neogen Customer and Technical Services can be contacted through neogen.com, and product training is available by request.

SDS Information Available

Safety data sheets are available for all test kits at neogen.com or by calling 800.234.5333 or 517.372.9200.

Terms and Conditions

Neogen's full terms and conditions are available online.

Warranty

Neogen makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. If any materials are defective, Neogen will provide a replacement of the product. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. Neogen shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.