

PRECAUTIONS

1. Samples intended to be tested for casein must be extracted separately from samples intended to be tested for other food allergens, such as peanut and egg residues. The extraction additives for each type of test are designed specifically for the target food allergen.
2. The controls and extraction reagents of the Veratox for Casein Allergen test kit may contain one or more of the following potentially allergic materials: milk, egg protein, peanut protein, soy protein, or tree nut protein. If allergic to any of these compounds, please use caution when using this product.
3. Concentrated food additives, colors and flavors may cause interferences with ELISA test methods. Contact Neogen Technical Services for validation information.
4. Hydrolyzed and fermented proteins may not be detected using ELISA methods for allergen testing. Due to the nature of the proteins, it may be undetectable in the assay, but there could still be active allergenic protein residue present.
5. If testing infant formula, contact Neogen for additional information.
6. Disposable extraction bottles and tubes must be used to extract casein samples to avoid cross-contamination.
7. The testing area must be totally free of milk products. A minute amount of milk protein in the environment can affect test results.
8. Store test kit between 2-8°C (35-46°F) when not in use. Do not freeze test kits.
9. Do not use kit components beyond expiration date.
10. Sample extracts must be cooled to room temperature (18-30°C, 64-86°F) prior to use.
11. Bring kits to room temperature (18-30°C, 64-86°F) prior to use.
12. Do not mix reagents from one kit with reagents from a kit with a different serial number.
13. Do not run more than 24 wells per test.
14. Follow proper pipetting techniques (e.g., prime tips and use clean tips).
15. Use only incubation times specified. Others may give inaccurate results.
16. Avoid prolonged storage of kits at ambient temperatures.

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 it is needed.
2. **Extraction solution:** Prepare extraction solution by adding a foil pouch of extraction solvent (10mM PBS, to 1 L distilled or deionized water at pH 7.4). Cover and store unused portion refrigerated (2-8°C, 35-46°F).
3. **Wash buffer:** Prepare wash buffer solution by mixing 40 mL of wash buffer concentrate in a wide-mouth bottle into 960 mL of distilled or deionized water at pH 7.4. Cover and store unused portion refrigerated (2-8°C, 35-46°F).
NOTE: Discard unused portions of extraction solution and wash buffer when test kit has been used completely.
4. **Antibody wells:** Keep wells sealed in the foil pouch until needed. Remove wells from the 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.

NOTE: Glassware and material used for peanut, almond and egg allergen testing cannot be used for milk residue testing due to the potential of cross-contamination. For this reason, it is also highly recommended that any labware, such as wash bottles, graduated cylinders, and 1 L bottles, be solely dedicated for use with the milk allergen kit.

1. Prepare the extraction solution (PBS, pH 7.4) as described in the procedural notes.
2. Preheat 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 an appropriate 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 250 mL disposable plastic extraction bottle.
5. Add 1 level scoop of the extraction additive into the sample bottle. (Do not use the extraction additive from another allergen test kit.)
6. Pour 125 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.
8. Extract by shaking (150 rpm) in a 60°C water bath for **15 minutes**. Remove the bottle from the bath.
9. Let the material settle for **5 minutes** before proceeding to the next step.
10. Use the supernatant (the top liquid portion of the extract) as the sample. Do not filter. Begin the test procedure once the sample has cooled to room temperature (at least **15 minutes**).

TEST PROCEDURE

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

1. Mix each reagent by swirling the reagent bottle prior to use.
2. 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. Run only two 12-well strips at a time.

0	2.5	5	10	15	S1	S2	S3	S4	S5	S6	S7
S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19
3. 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 microwell holder back and forth on a flat surface.
4. Incubate microwells **10 minutes** at room temperature (18-30°C, 64-86°F). Discard the red-marked transfer wells.
5. 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 10 times, then turn the wells upside down and tap out on a paper towel until the remaining washing solution is removed.
6. Pour the needed volume of conjugate from the blue-labeled bottle into a clean reagent boat.
7. Using the 12-channel pipettor, transfer 100 µL of the conjugate into all the wells and 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. Wash all wells with the wash buffer solution as described in step 5.
10. Pour the needed volume of substrate solution from the green-labeled bottle into a clean reagent boat.
11. Place new tips on the 12-channel pipettor and transfer 100 µL of substrate into each well and mix for **20 seconds** by sliding the microwell holder back and forth on a flat surface.
12. Incubate for **10 minutes** at room temperature (18-30°C, 64-86°F).
13. Pour the needed volume of Red Stop solution from the red-labeled bottle into a clean reagent boat.
14. Place new tips on the 12-channel pipettor and transfer 100 µL of Red Stop into each well and mix for **20 seconds** by sliding the microwell holder back and forth on a flat surface.
15. Wipe the bottom of the microwells with a dry cloth or towel 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.
16. Read and calculate the test's results using Neogen's Stat Fax microwell reader, or an equivalent strip reader. If using a strip reader, calculate the results using Neogen's Veratox software for Windows.

PERFORMANCE CHARACTERISTICS

Limit of quantitation: 2.5 ppm (described as the lowest concentration point on the calibration curve that this test can reliably detect casein allergen.)

Range of quantitation: 2.5 – 15 ppm (for quantitating samples above 15 ppm, contact a Neogen representative for dilution instructions.)

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

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

CUSTOMER SERVICE

Neogen Customer Assistance and Technical Services can be reached by using the contact information on the back of this booklet. Training on this product, and all Neogen test kits, is available.

MSDS INFORMATION AVAILABLE

Material safety data sheets (MSDS) are available for this test kit, and all of Neogen's test kits, on Neogen's website at www.neogen.com, or by calling Neogen at 800/234-5333 or 517/372-9200.

WARRANTY

Neogen Corporation 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.

TESTING KITS AVAILABLE FROM NEOGEN

Natural toxins

- Aflatoxin, DON, ochratoxin, zearalenone, T-2/HT-2 toxins, fumonisin, histamine

Foodborne bacteria

- *E. coli* O157:H7, *Salmonella*, *Listeria*, *Listeria monocytogenes*, *Campylobacter*, *Staphylococcus aureus*, *Salmonella enteritidis*

Sanitation

- ATP, yeast and mold, total plate count, generic *E. coli* and total coliforms, protein residues

Food allergens

- Almonds, crustacea, eggs, gliadin, hazelnut, lupine, milk, mustard, peanut, sesame, soy, walnut

Genetic modification

- CP4 (Roundup Ready®)

Ruminant by-products

- Meat and bone meal, feed



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Read instructions carefully before starting test



Casein Allergen Quantitative Test

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

CASEIN 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 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 milk being one of the most prevalent.

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

INTENDED USE

Veratox for Casein Allergen is intended for the quantitative analysis of food products, such as juices, sauces and sorbets, or environmental surfaces for the presence of casein.

INTENDED USER

The Veratox for Casein Allergen test kit is designed for use by quality control personnel and others familiar with foods possibly contaminated by casein or milk products. Since technique is very important, operators should be trained by a Neogen representative or someone who has successfully completed the Neogen training.

ASSAY PRINCIPLES

The Veratox for Casein Allergen test is a sandwich enzyme-linked immunosorbent assay (S-ELISA). Casein residue is extracted from samples with a buffered salt solution (Phosphate Buffered Saline, or PBS) by shaking in a heated water bath. Extracted casein residue is sampled and added to antibody-coated wells (capture antibody) where it binds to the antibody during an incubation. Any unbound residue is washed away and a second antibody (detector antibody), which is enzyme labeled, is added. The detector antibody binds to the already bound casein residue. 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 (24 per pouch)
2. 48 red-marked transfer wells (24 per pouch)
3. 5 yellow-labeled bottles of 0, 2.5, 5, 10, 15 ppm nonfat dry milk (NFDM) controls
4. 4 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 foil pouches of 10 mM PBS dry powder extraction solvent (each pouch contains enough powder to prepare 1 L of extraction solvent)
8. 2 widemouth bottles of 40 mL PBS-Tween wash buffer concentrate (each bottle contains enough concentrate to prepare 1 L of wash buffer)
9. 50 g 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 disposable transfer pipettes
2. Shaker water bath adjusted to 60°C ± 1° (140°F) with clamps to hold extraction bottles
3. Adjustable 50-200 µL pipettor (Neogen item 9276)
4. Pipette tips (Neogen item 9410)
5. 12-channel pipettor (Neogen item 9273)
6. Timer (Neogen item 9426)
7. Microwell strip reader with a 650 nm filter (Neogen item 9303)
8. 1 L bottle to prepare washing solution (Neogen item 9472)
9. 1 L heat safe bottle to prepare extract solution (Neogen item 9472)
10. Paper towels or equivalent absorbent material
11. Microwell holder (Neogen item 9402)
12. Waterproof marker
13. Wash bottle (Neogen item 9400)
14. Distilled or deionized water
15. 3 reagent multichannel pipettor boats (Neogen item 9435)
16. Graduated cylinder capable of measuring 125 mL (Neogen item 9368)
17. Scale capable of weighing 5 g (Neogen item 9435)