

Neogen Europe Ltd., in cooperation with the University of Nebraska's
Food Allergy Research and Resource Program (FARRP) present

Food Allergen Handbook



NINTH EDITION

 **NEOGEN**
Europe Ltd.

food allergy research
& resource program
farrp

Neogen Corporation develops and markets products and services dedicated to food and animal safety. The company's Food Safety Division markets diagnostic test kits to detect foodborne bacteria, natural toxins, genetic modifications, food allergens, drug residues, plant diseases and sanitation concerns. These diagnostic test kits are less expensive, easier to use, and provide greater accuracy and speed than many of the conventional diagnostic methods currently employed. Neogen's Acumedia subsidiary has been a premier manufacturer of dehydrated culture media since 1978. For more information, please call +44 (0)1292 525 600.

The University of Nebraska's Food Allergy Research and Resource Program (FARRP) is a part of the Department of Food Science and Technology. FARRP is a food industry and university partnership which was formed to provide research and resource tools for the food industry in the area of food allergens. It is the leader in training and educating the industry on allergen awareness. For more information, please call 001-402/472-4484.

NEOGEN'S FOOD ALLERGEN TESTS

Almond	8440	Veratox for Almond Allergen
	8441	Alert for Almond Allergen
Crustacea	902086G	Reveal 3-D Almond
	8520	Veratox for Crustacea
Egg	902081S	Reveal 3-D Crustacea
	8450	Veratox for Egg Allergen
	8451	Alert for Egg Allergen
Gliadin/ Gluten	902082Q	Reveal 3-D Egg
	8480	Veratox for Gliadin
	8481	Alert for Gliadin
	8510	Veratox for Gliadin R5
	8511	Alert for Gliadin R5
	8519	Reveal 3-D Gliadin
Hazelnut	901031P	Reveal 3-D Gluten
	8420	Veratox for Hazelnut Allergen
Lupine	902087E	Reveal 3-D Hazelnut
	8500	Veratox for Lupine Allergen

Milk	8470	Veratox for Total Milk Allergen
	8471	Alert for Total Milk Allergen
	8479	Reveal 3-D for Total Milk Allergen
	902061Y	BioKits BLG Assay
Mustard	8460	Veratox for Casein Allergen
	8400	Veratox for Mustard Allergen
	8405	Reveal 3-D for Mustard Allergen
Peanut	8430	Veratox for Peanut Allergen
	8431	Alert for Peanut Allergen
	8438	Reveal for Peanut Allergen
	901041L	Reveal 3-D Peanut
Sesame	902070X	BioKits Sesame Assay
	8535	Reveal 3-D Sesame
Soy	8410	Veratox for Soy
	8411	Alert for Soy
	902093K	Reveal 3-D Soy
Walnut	902085J	BioKits Walnut Assay

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WHAT IS FOOD ALLERGY?

A recent review showed most studies found that clinically proven allergy to any food occurs in 1–5% of the total population in Europe. Many believe that the number of people with a food allergy is increasing. It is also an impression gained by doctors running allergy clinics that the occurrence of food allergens changes with age and varies across different geographical areas. Clinical evidence suggests that fruits, vegetables, tree nuts, and peanuts are responsible for most allergic reactions among adults while milk and egg allergies are the most common kind of allergy in children.

Food allergens are proteins in some foods that can trigger an immune response in allergic individuals. Current experience indicates that an allergic response can be triggered by eating a food containing minute quantities of a food allergen, with the specific amounts needed to trigger the response varying from individual to individual.

Once ingested, food allergens can cause a number of symptoms, ranging from mild hives to severe gastrointestinal and respiratory symptoms, including nausea, vomiting, throat swelling, asthma and trouble breathing. The most serious food-allergic reaction is anaphylactic shock, which is a severe shock reaction that can include any of the symptoms previously described, but also includes a dangerous drop in blood pressure and sometimes cardiac arrhythmia. Anaphylactic shock can be life-threatening if not treated immediately.



WHY TEST FOR FOOD ALLERGENS?

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

Testing also can add to, and protect, a company's reputation. Currently, some companies use a precautionary statement such as, "may contain peanut and peanut products" on the ingredient label, even though there is very little chance the product actually contains any peanut. If testing is done, companies may be able to minimise the use of precautionary labels.

In companies that use push-through product to clean equipment between products, testing can allow the company to determine exactly how much push-through product is necessary to achieve the level of cleanliness necessary for food allergens. Testing can eliminate guesswork, and save product from going to waste or from having to be reworked. Testing clean-in-place (CIP) solutions, final product, and certain equipment after the sanitation crew has finished, can identify sources of cross-contact, and also verify cleanliness before changeover.

The most obvious reason for testing is to protect a company from staggering costs. If a product contains undeclared, potentially hazardous allergens, the company would contact the government and initiate a voluntary recall. Product recalls can cost food companies millions of Euros.

THE FOOD ALLERGEN LABELLING DIRECTIVE OF 2007

The labelling directive (Directive 2000/13/EC) and its later amendments is an important piece of EU legislation that specifically refers to allergenic foods. The labelling directive requires manufacturers to declare all ingredients in pre-packaged foods sold in the EU with very few exceptions. This directive has been amended a number of times with regard to allergens. Directive 2007/68/EC has the most recent amendment of Annex IIIa. It lists all the allergenic food that must be labelled when present in a product as well as a few products derived from these foods for

which allergen labelling is not required. EU regulations require food manufacturers to label 14 known ingredients that can trigger an allergic reaction if they are contained within the recipe of the food: cereals, crustacean, fish, egg, peanut, soybean, milk, nuts, celery, mustard, sesame seeds, sulphur dioxide, lupine, molluscs.

WHY TEST FOR GLIADIN AND OTHER PROLAMINS?

Gliadin and other prolamins have been identified as major causal agents in a number of disorders, including wheat allergy and gluten intolerance (Coeliac disease). Wheat allergy is a specific immune response to a number of wheat proteins, including gliadin, albumin, globulin, and glutenin. Coeliac disease is a chronic reaction to gluten proteins that results in the poor absorption of nutrients in the small intestine.

Gliadin is an alcohol-soluble protein found in wheat that belongs to a group of proteins called prolamins. Other prolamins include secalin, found in rye, and hordein, found in barley. Neogen's tests for gliadin also detect secalin and hordein. Gluten consists of two groups of proteins (prolamins and glutelins) that are found in differing amounts in wheat, barley, rye and oats. Since gliadin represents approximately 50% of gluten, a Neogen Veratox result of 10 ppm of gliadin would correspond to 20 ppm gluten.



Those with wheat allergy or coeliac disease must avoid gluten, and rely upon the correct labelling of food to make appropriate, safe food choices. Testing for the presence of gluten components ensures food manufacturers an unlabelled—and potentially dangerous—ingredient did not make its way into a food product.

In addition to the wheat allergen implications, food companies labelling a product as gluten-free must ensure that their product meets this claim. The 2008 revision of The Codex Alimentarius defines “gluten-free” as products with a gluten level that does not exceed 20 ppm (10 ppm gliadin). In Europe, EU regulation No 41/2009 requires that only foods that do not exceed 20 parts per million (ppm) gluten will be able to be labelled ‘gluten-free’. Where certain foods have been specially treated, such as gluten containing cereals which have been treated and contain less than 100 ppm gluten, they may be labelled ‘very low gluten’.

HOW DO RAPID TESTS HELP PREVENT ALLERGEN CROSS-CONTACT?

Rapid food allergen test kits give a company a method of easily determining if its product has been subjected to cross-contact and an investigative tool to determine how and when the cross-contact occurred. Companies can use the test kits on raw material before it enters production or on equipment or product at any point throughout the production process. The tests' flexibility and ease of use allow users to pinpoint and eliminate possible risks for cross-contact.

SCREENING VS. QUANTIFYING RESULTS

Neogen's rapid tests for the detection of food allergens and gliadin are available in multiple formats. Neogen's Reveal® and Reveal 3-D allergen screening tests are simple strip tests with results in less than 10 minutes following sample extraction using Reveal and only five minutes after extraction with Reveal 3-D. The company's screening line of microwell tests, Alert®, provides easy-to-interpret visual results. Neogen's quantifying tests, Veratox® and BioKits, use a microwell reader and preset calculations (programmed into the reader or a computer) to determine exact concentrations of target allergens.

A. Screening tests

Neogen's line of screening tests allow for the rapid determination of the presence of a target food allergen on environmental swabs and in some food products.

C. BioKits (BLG)

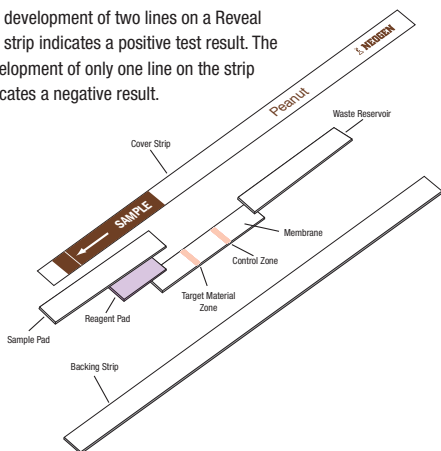
The BLG assay is an indirect competitive enzyme-linked immunoassay. The target food allergen protein is extracted from samples. Extracted protein is sampled and added to antibody-coated microwells, where it binds to the antibody during an incubation. Biotinylated antibody is added after the initial incubation and allowed to incubate again. A wash step is performed, then conjugate is added and binds to the already bound biotinylated antibody. After a second wash, substrate is added. Colour develops as a result of the presence of bound conjugate. Stopping solution is added and the microwells are read in a microplate reader fitted with a 450 nm filter.

D. Reveal (peanut)

Neogen's Reveal format for the detection of food allergens is a single-step lateral flow immunochromatographic assay. The extract is wicked through a reagent zone, which contains antibodies specific for the target allergen conjugated to coloured particles. If allergen is present, it will be captured by the conjugated antibodies. The allergen-antibody-particle complex then is wicked onto a membrane which contains a zone of antibody specific for the target allergen. This zone captures the complex allowing the particles to concentrate and form a visible line. If no target allergen is present, no line will form. The membrane also contains a control zone where an immune complex present in the reagent zone is captured by an antibody, forming a visible line. The control line always will form regardless of the presence of the target allergen, ensuring the strip is working properly.

Reveal Food Allergen Screening Tests

The development of two lines on a Reveal test strip indicates a positive test result. The development of only one line on the strip indicates a negative result.

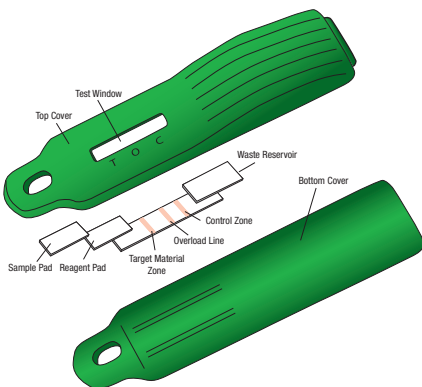


E. Reveal 3-D

Neogen's Reveal 3-D format for the detection of food allergens is a single-step lateral flow immunochromatographic assay. The extract is wicked through a reagent zone, which contains antibodies specific for the target allergen conjugated to coloured particles. If allergen is present, it will be captured by the conjugated antibodies. The allergen-antibody-particle complex then is wicked onto a membrane which contains a zone of antibody specific for the target allergen. This zone captures the complex allowing the particles to concentrate and form a visible line. If no target allergen is present, no line will form. In addition, the 3-D format has a unique feature to guard against oversaturation. The second line referred to as the "overload line" will disappear in situations where gross contamination is observed preventing false negative results for occurring. The membrane

Reveal 3-D Tests

Neogen's Reveal 3-D tests are uniquely designed with 3 lines of detection. Positive results will show a line at position T, O and C. No line will appear at T and lines present at O and C indicates below detection limit. If no line is visible at position O, and if a line is faintly visible or absent at T, the sample is overloaded with target material.



also contains a control zone where an immune complex present in the reagent zone is captured by an antibody, forming a visible line. The control line will always form regardless of the presence of the target allergen, ensuring the strip is working properly.

LIMITATIONS OF ELISA-BASED FOOD ALLERGEN TESTS

ELISA-based food allergen tests, like Neogen's, are not appropriate for use in certain applications. Because the tests are based on an antibody reaction with an extracted allergenic protein, the protein in the sample must be close to its natural state and readily extractable. Although this normally is the case, in certain instances the test may not yield results totally indicative of the sample's potential to produce an allergic reaction in susceptible consumers. The user cannot assume that if a protein is undetectable it is not allergenic. Some of these instances include (but are not limited to):

- Hydrolyzed and proteolyzed proteins (e.g., HVP, hydrolyzed egg protein)
- Fermented products and cultures (e.g., guar gums, xanthan gums, soy sauce)
- Probiotic cultures
- Enzyme proteases
- Some concentrated food additives, colours and flavors
- Some oil-based ingredients (e.g., oil, lecithin, oil-soluble flavors, etc.)

SAMPLING INGREDIENTS, PRODUCTS, LIQUIDS AND RINSES

Neogen's experience has shown the vast majority of errors associated with food testing can be attributed to how the original sample was obtained. Taking steps to ensure the sample to be tested is representative of the product as a whole will increase confidence in subsequent test results.

What follows are generally recommended guidelines for ingredient and product sampling, according to material type. Please contact Neogen with questions about the adaptability of the guidelines to specific testing needs.

A. Dry, blended or finished ingredients and products

1. Obtain a 500 g sample from the ingredient or product to be tested and place in a clean container.
2. Thoroughly mix/blend the 500 g sample with a clean spatula or blender for at least **30 seconds**.
3. Remove a 50 g subsample from the 500 g sample.
4. If the product has a large particle size, place the 50 g in a grinder and grind to a very fine particle size.
5. Thoroughly mix/blend the subsample with a spatula or blender for at least **30 seconds**.
6. From the 50 g, remove the appropriate size sample for testing with one of Neogen's food allergen test kits. **NOTE:** Neogen recommends that the remainder of the sample be saved for confirmatory testing should a food allergen be detected.
7. Thoroughly clean the grinder/blender and utensils between samples.

B. Liquids and CIP rinses

For homogenous liquids, it is not necessary to sample a large quantity. Simply draw the sample from the product or rinse to be tested for use with one of Neogen's food allergen test kits, and add to the extraction solution.

NOTE: Neogen recommends that at least 10 mL of the product or rinse be saved for confirmatory testing should a food allergen be detected.

ENVIRONMENTAL SAMPLING AND EXTRACTION

Environmental sampling for food allergen detection should be performed after equipment has been thoroughly cleaned, and before production of the following lot has begun. Because environmental sampling requires the

use of swabs and a specialized extraction of possible allergens from the swabs. Neogen recommends the use of the Allergen Environmental Swabbing Kit (Neogen item 8432S) for use with one of Neogen's Veratox, Alert or Reveal food allergen test kits. The BioKits Allergen Swabbing Kit (BASK, Neogen item 901042J) is recommended for collecting environmental swabs for use with the BioKits Assay test kits. The Reveal 3-D food allergen test kits are supplied with swabs for sample collection.

If you are not using one of Neogen's environmental swabs, alternate swabs should be tested to ensure they do not cross-react with Neogen's food allergen test kits. Do not use swabs intended for microbial sampling that contain growth media. Do not use sponges.

Whether or not the Neogen swabs are used, sampling should include areas known to be hard to clean in the environment to be tested. These may include equipment and conveyor nooks and crevices, scarred work surfaces, or any area where food residue buildup is a known concern.

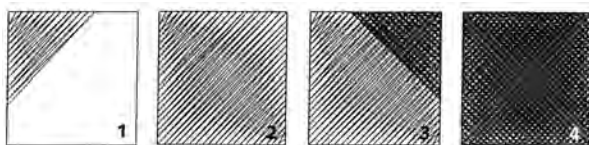
Neogen recommends the following procedure for environmental testing:

A. Prepare extraction solution

Prepare the appropriate extraction solution per the instructions provided in the specific food allergen test kit.

B. Swabbing

1. Gather the sample with a swab, using one of the following methods:



- a. For dry surfaces: Open a new swab and wet with extraction solution. Swab a 10 x 10 cm area by using the crosshatch technique (see above).
- b. For wet surfaces: Open a new swab and swab a 10 x 10 cm area by using the crosshatch technique. Do not moisten swab prior to use.

NOTE: When testing equipment, the swabbing area should be chosen carefully. Areas to swab should be potential food hang-up areas, such as scarred surfaces, corners, angles, and any known food buildup area. An effective swabbing procedure, such as the crosshatch technique (illustrated previously), should be used to ensure detection of any present allergen proteins by covering virtually 100% of the chosen area.

2. Return the swab to its appropriate tube once sampling is complete. Remember to label each tube. Refer to the kit instructions for final extraction of the swab prior to analysis.

HOW CAN ALLERGEN VERIFICATION FIT INTO A FOOD SAFETY PROGRAM?

Allergen verification can be included as a sanitation validation in a company's food safety program, or as part of a sanitation standard operating procedure (SSOP) when changing over from an allergen-containing product. Since allergens are classified as a chemical hazard by the FDA, with the unlabelled allergen being the hazard, it is imperative to have an allergen control plan in place. Examples of possible strategies to control allergens in a food manufacturing facility include:

A. Sanitation verification and validation using environmental swabs

1. Swab equipment surfaces prior to cleaning to assess the level of allergen present.
2. Clean production line according to the SSOP.
3. Swab equipment surfaces again to assess the level of allergen remaining.

4. Reclean if necessary.
5. Send non-allergen product through the equipment and test the first product produced. Test a representative number of samples throughout the first lot of product.
6. If the testing shows that the SSOP is adequate, then precautionary labelling should not be necessary. Exceptions may exist for particular allergen materials. In such cases more testing may be necessary.
7. All test product should be placed on hold or discarded.

B. Allergen verification as part of a food safety program

1. The production of safe food products requires that the food safety program be built upon a solid foundation, which may include, but are not limited to:
 - a. **Supplier controls.** Suppliers should be asked to provide information on their allergen prevention programs.
 - b. **Specifications.** All incoming ingredients should conform to specifications consistent with allergen control programs.
 - c. **Education.** All personnel should be trained about food allergen concerns.
 - d. **Product identification.** Product identification, traceability and recall procedures should be in place for all products produced.
 - e. **Good Manufacturing Practices (GMPs).** Examples of GMPs include:
 - Design of equipment for easy cleanup
 - Sanitation standard operating procedures (SSOPs)
 - Sanitation and control of receiving and storage areas
 - Sanitation and control of distribution points
 - f. **Identification of allergen sources.** Potential allergen sources include:
 - Raw materials
 - Ingredients
 - Sub-ingredients, e.g., natural flavours
 - Rework-processing aids, e.g., wheat starch
 - Packaging materials
 - Cross-contact with shared equipment
2. Allergen verification can be quickly and easily performed using Neogen's screening and/or quantitative testing products. Specific areas that can be monitored include:
 - a. **Incoming ingredients.** Suppliers should have an allergen control program verified by first off testing to ensure their products are accurately labelled.
 - b. **Environmental swabs.** Swabs should be run after cleanup, and prior to the next production run. Any positive samples could indicate inadequate cleaning, and recleaning should be performed.
 - c. **CIP solutions.** A portion of the final CIP rinse solution can be tested. Any positive results may indicate inadequate cleaning, and additional steps may be required. Rinse solutions are very dilute so confirmation by finished product testing is recommended.
 - d. **Hazard analysis.** For initial identification of an allergen hazard during the risk assessment, a quantitative level of allergen is needed. Examples include assessing the undeclared allergen levels in push-through product, final product, etc.

EXAMPLE: ALLERGEN VERIFICATION IN A FOOD SAFETY PROGRAM

Company A, a bakery manufacturer, produces three flavours of cookies in the same facility: vanilla, sugar, and peanut butter. As part of its allergen control plan, Company A asks its supplier to certify non-peanut ingredients are peanut-free. To further minimise the risk of peanut contamination, Company A follows the following system as a part of its overall food safety program:

A. Testing of raw material

Every 5th load of incoming raw material is tested for peanut allergen using the Alert for Peanut Allergen screening test, and delivery is not accepted until a negative test result is received. In the event of a positive sample, the delivery is either rejected or rerouted.

B. Testing of food contact surfaces

After the peanut butter cookies are run, the processing equipment is cleaned. After the cleanup step, but prior to the next production run, each piece of equipment is swabbed, and swabs are screened for peanut residue. In the event of a positive result, the line is re-cleaned and retested.

C. Quantitative testing

In instances where a quantitative level of peanut is needed (e.g., to determine the effect of the ingredient in a final product), the Veratox test kit is used.

RECOMMENDED TEST POINTS FOR VALIDATION OF ALLERGEN CONTROL STRATEGIES

A. Sanitation

1. All food contact surfaces which have come into contact with an allergen at some point.
2. Totes, pails, etc., used to transport allergens, if not dedicated.
3. All cleaning utensils used to clean production equipment where an allergen has been run, (e.g., brushes, rags, scrubbers, dust collectors, etc.), if not dedicated.
4. All sampling devices used to draw samples from an allergenic run.
5. Any push-through product used to clean-out prior to allergenic product.
6. All rework (if not from a like product).
7. Final CIP rinse.

B. Ingredients and raw materials

1. Any ingredient or sub-ingredient derived from an allergenic source (unless verified information from supplier indicates no issues), or those that may have come into contact with an allergen.
2. All natural and artificial flavourings, spices and additives which may be derived from an allergenic source, or have come into contact with an allergen (unless verified information from a supplier indicates no issues).
3. Any product not previously tested where a change in ingredient or formulation has been made.

C. Finished and in-process product

1. First product after changeover from allergenic to non-allergenic product (gluten to gluten-free product).
2. Products where “may contain...” or similar labelling is used, to justify use of the statement.
3. Investigating food allergic consumer complaints about finished products.
4. All non-allergenic in-process product which has followed an allergenic run.



FOOD ALLERGEN SELF-EVALUATION CHECKLIST

A. Prior to initiation of a food allergen verification program

- 1. Are your staff, including part-time and temporary employees, trained on the severity of food allergies and the impact of recalls due to undeclared food allergens?
- 2. Is there a clear and defined labelling strategy, including:
 - a. Use of simple and everyday terms (e.g., “milk” instead of “calcium caseinate”)?
 - b. Is “may contain...” or similar labelling used only after thorough GMPs and SSOPs have been created and followed with respect to all allergens?
 - c. Is “may contain...” or similar labelling used only when the food allergen cross-contact is uncontrollable, sporadic and documented?
- 3. Have suppliers and co-packers been included in your food allergen control plan?
 - a. Have allergen questionnaires on ingredients been sent to all suppliers and received back?
 - b. Are there supplier/co-packer audits in place?
 - c. Are suppliers and co-packers aware of your food allergen control expectations?
- 4. Is there a clear and defined recall strategy in place?
- 5. Are there clear and defined consumer response strategies in place?

B. System design and product formulation

- 1. Is production equipment designed for easy, thorough cleaning, allowing the complete cleaning of filler heads, valves, belts and other equipment as necessary?
- 2. Have allergenic products been introduced into the production process at the latest possible stage of production?
- 3. Have allergenic products been scheduled at the end of a production shift, prior to clean-up?
- 4. If a non-allergenic product is following an allergen containing product in production, is a full allergen clean-up done?
 - a. Are all food contact surfaces “visibly clean” (no visible product remains)?
 - b. Is the “allergen clean” validated?
 - c. Is there a routine cleaning procedure verification program in place?

QUESTIONS AND ANSWERS REGARDING FOOD ALLERGEN TESTING

1. What is the difference between the terms “milk” and “total milk”?

“Milk” is a general term for a product that may or may not include both of the major dairy proteins—whey and casein. Since both casein and whey can be allergenic, Neogen uses the term “total milk” for its test to indicate that both casein and/or whey can be detected using the same test kit.

2. What is the relationship between “gliadin” and “gluten”?

Gluten is the major protein in wheat, rye, and barley. Gliadin is one of two prolamins of gluten that make up the gluten protein. The total protein content of gluten is approximately 50% gliadin. To determine the gluten content in a sample from a Neogen test for gliadin, simply multiply the sample’s test result by two (10 ppm gliadin = 20 ppm gluten).

3. Can a blender method be used for extraction?

Yes, a simple blender extraction method has been validated for use with Neogen’s screening tests (Alert and Reveal) for almond, peanut, egg, hazelnut, and soy. The blender extraction method does not yield acceptable results when used with Neogen’s screening tests for total milk or gliadin, or any of Neogen’s quantitative Veratox food allergen tests. All Veratox allergen tests, with the exception of gliadin with its unique extraction protocol, require the use of a shaker water bath method for sample extraction.

4. Can I make my Veratox or Alert allergen test kit more sensitive?

The detection limits of Neogen’s test kits have been carefully set based on the most practical levels as determined by the food industry, regulatory and expert consultants. However, there may be instances where a lower level of detection is needed. In most cases, this can easily be accomplished. Contact a Neogen representative for specific applications and procedures.

5. Can lab cleanliness affect sample results?

Laboratory conditions can affect test results. Neogen tests are designed for on-site testing; however, they are extremely sensitive and can unintentionally detect allergenic proteins that may exist in laboratory environment. Therefore, it is highly recommended that the sample preparation and testing areas, and all instruments, be regularly cleaned.

6. How long can sample extracts and swabs be stored before testing?

Once extracted, samples from swabs should be evaluated within **4 hours**. All other sample extracts should also be tested within **4 hours**.

7. Where are the best locations to sample the environment with swabs?

To yield test results that reflect true environmental conditions, samples should be taken not just from food contact surfaces, but also from corners, scarred work areas, screw heads, and any other areas where there is potential for food hang-up.

8. When should you test clean-in-place (CIP) rinse solutions?

In some closed systems where environmental sampling is not possible with a swab, CIP final rinses may be the only other option than product testing for verifying sanitation cleanliness.

9. Why is ATP testing not effective for food allergen monitoring?

ATP (adenosine triphosphate) is a substance in all organic matter, living or dead, and hence is not specific

enough for allergen verification. No matter how sensitive the ATP tests claim, there is no way to differentiate ATP from an allergenic food from that of all other sources of ATP. Also, many allergenic foods contain very low levels of measurable ATP, which would cause potential false negative results if testing for a food allergen using an ATP method.



10. How does heat processing affect the recovery of food allergens on the Neogen test kits?

Neogen has carefully designed its test kits to accommodate heat processed and highly processed samples. In some cases (e.g., its gliadin test), a special additive is used for processed samples. In rare cases highly refined proteins may not be detectable (see question 11).

11. Are Neogen’s allergen test kits appropriate for all samples?

Food allergens cannot be detected in some specific sample types by any commercially available test kit. Fermented and hydrolysed proteins, as well as fermentation substrates such as gums, may not be detected due to the nature of the proteins, but there still could be active allergenic protein residue present. Food allergens also may not be detectable in some concentrated food additives, colours, and flavours. Contact Neogen if you have a question about a specific commodity.

12. What is the role of the “additive” in the extraction process?

An extraction additive plays two roles simultaneously—it enhances the solubility and stability of the allergenic protein, and eliminates background interference contributed by the food matrix being tested.

13. Are there analytical confirmatory methods for food allergen testing?

Currently, no confirmatory methods for food allergens exist. However, for confirmation of test results, there are many third party laboratories that run full, quantitative ELISA methods or PCR methods and some laboratories that run LC MS/MS.

14. Why are the Alert and Reveal test kits set at 5 ppm and 10 ppm?

Because regulators have not set actual thresholds for allergens, the food industry has taken a proactive approach of self-governance and chosen these levels. They are relevant by minimising risk to the consumer, without going to “zero tolerance” as a threshold.

15. What does the term “limit of detection” mean? Is it different than “limit of quantitation”?

A test kit’s limit of quantitation (LOQ) refers to the lowest point that its results are quantifiable. As a general rule, ELISAs identify this as the first non-zero control in that kit, which is the 2.5 ppm control in Neogen’s test kits. The limit of detection (LOD) is the lowest point at which a result can be considered above background noise. A LOD is determined as the mean of 10 evaluations of a known negative sample, plus two standard deviations. However, results greater than the LOD but less than the LOQ are analytically valid for qualitative purposes only.

16. Should a sample that tests below 5 ppm on an Alert kit be called “negative”?

No. As indicated above, the 5 ppm level has been established and generally recognised by the food industry as an indicator of risk associated with testing. However, levels below 5 ppm do not mean the sample is negative, as levels between the detection limit and 5 ppm may still be allergenic. More appropriate would be the phrase: “Below limit of detection (BLD)”.

17. What are the allergen kits detecting and how is it reported?

Each specific test kit detects the presence of residue from the allergenic food of concern. Each test reports the ppm value to the total allergenic food. For example: peanut results are reported as ppm of total peanut, as opposed to protein only. See Appendix A for specific allergen information. See Appendix D for converting allergenic food results to protein results.

18. Are all manufacturers’ food allergen test kits reporting on the same scale?

No, some manufacturers report results in ppm protein, others in ppm total allergenic food. Neogen’s Veratox and Alert tests report as the ppm of total allergenic food. Those who prefer to convert their results from ppm total allergenic food to ppm allergenic protein can do so based on average protein content of these foods. For example, nonfat dried milk (NFDM) contains approximately 35% protein. Therefore, a 5 ppm total milk on the Veratox for Total Milk Allergen test would be approximately 1.75 ppm milk protein. Peanut is approximately 26% protein, so 5 ppm Veratox for Peanut results would convert to 1.3 ppm protein. See Appendix D for converting allergenic food results to protein results.

19. How many ppm can be detected from a rinse?

In general, the sensitivity for the 3-D test kits for rinses ranges from 5–10 ppm; however, this can fluctuate based on the type and concentration of cleaning chemicals and sanitizers found in the rinse water. It is recommended to only test final rinse waters since some chemicals and sanitizers may have an effect on the tests.

20. How many ppm can be detected from a swab?

It is not possible to discuss sensitivity from a surface swab as this type of analysis does not have a defined sample size. By definition, ppm is mg of contaminant per kg of sample. Because sample size widely fluctuates depending on the amount of material captured on the swabs surface, a ppm definition cannot apply. Instead it is appropriate to define sensitivity in terms of µg of contaminant found in a 100 cm² surface. (10 cm x 10 cm surface was used during validation). Given this definition, 5–10 µg of allergen per 100 cm² is achievable; however, this can vary depending on the surface and type and concentration of cleaning chemicals or sanitizers present on the surface.



Reveal for Peanut Allergen test kit

21. Can I test product on the Reveal 3-D kits?

Because of the infinite number of variables associated with ingredient sources and process changes contributing to the change in the limit of detection from that matrix, it is believed it is best to recommend microwell test kits for product applications as they will yield the most consistent results. This in no way invalidates previous validations performed for ingredients and products provided that the ingredient source and process remain unchanged from the time of validation.



Reveal 3-D for Peanut test kit

NEOGEN TEST KITS: USAGE AND APPLICATION

Overview:

Neogen manufactures the most complete line of rapid food allergen test kits on the market, including various formats to fit individual testing situations. To this end, Neogen has developed the following chart to assist in determining which test kit is most appropriate for your application. In general, the Veratox and BioKits tests are both quantitative test kits. The Alert format is a 24 well qualitative assay. Both the Reveal test and Reveal 3-D test kits are simple lateral flow tests designed primarily for environmental testing.

Neogen test kit and product number	Target ¹	Usage ²	Application ^{3,4}
ALMOND ALLERGEN			
8400 Veratox for Almond Allergen	Total almond protein	Quantitative	Products and ingredients
8441 Alert for Almond Allergen	Total almond protein	Qualitative: 5 ppm	Products, ingredients, swabs, rinses
902086G Reveal 3-D Almond Test	Total almond protein	Qualitative: low ppm	Swabs and rinses
CRUSTACEA ALLERGEN			
8520 Veratox for Crustacea Allergen	Total crustacea	Quantitative	Products and ingredients
902081S Reveal 3-D Crustacea Test	Total crustacea protein	Qualitative: low ppm	Swabs and rinses
EGG ALLERGEN			
8450 Veratox for Egg Allergen	Egg white	Quantitative	Products and ingredients
8451 Alert for Egg Allergen	Egg white	Qualitative: 5 ppm	Products, ingredients, swabs, rinses
902082Q Reveal 3-D Egg Test	Egg white	Qualitative: low ppm	Swabs and rinses
GLIADIN/GLUTEN			
8480 Veratox for Gliadin	Gliadin/gluten	Quantitative	Products and ingredients
8481 Alert for Gliadin	Gliadin/gluten	Qualitative: 10 ppm	Products, ingredients, swabs, rinses
8510 Veratox for Gliadin R5	Gliadin/gluten	Quantitative	Products and ingredients
8511 Alert for Gliadin R5	Gliadin/gluten	Qualitative: 10 ppm	Product, ingredients, rinses and swabs
8519 Reveal 3-D Gliadin	Gliadin/gluten	Qualitative: 5–10 ppm	Swabs and rinses
901031P Reveal 3-D Gluten Test	Gluten	Qualitative: low ppm	Non-heat processed swabs and rinses

Neogen test kit and product number	Target¹	Usage²	Application^{3,4}
HAZELNUT ALLERGEN			
8420 Veratox for Hazelnut Allergen	Total hazelnut protein	Quantitative	Products and ingredients
902087E Reveal 3-D Hazelnut Test	Total hazelnut protein	Qualitative: low ppm	Swabs and rinses
LUPINE ALLERGEN			
8500 Veratox for Lupine Allergen	Total lupine protein	Quantitative	Products and ingredients
MILK ALLERGEN			
8470 Veratox for Total Milk Allergen	Total milk protein	Quantitative	Products and ingredients
8471 Alert for Total Milk Allergen	Total milk protein	Qualitative: 5 ppm	Products, ingredients, swabs, rinses
8479 Reveal 3-D for Total Milk Allergen	Total milk (casein/whey)	Qualitative: 5–10 ppm	Swabs, rinses, liquid foods
902061Y BioKits BLG Assay	BLG	Quantitative	Products and ingredients: large batch sizes
8460 Veratox for Casein Allergen	Casein	Quantitative	Products and ingredients: large batch sizes
MUSTARD ALLERGEN			
8400 Veratox for Mustard Allergen	Total mustard protein	Quantitative	Products and ingredients
8405 Reveal 3-D for Mustard	Total mustard	Qualitative: 5 ppm	Swabs and rinses
PEANUT ALLERGEN			
8430 Veratox for Peanut Allergen	Total peanut protein	Quantitative	Products and ingredients
8431 Alert for Peanut Allergen	Total peanut protein	Qualitative: 5 ppm	Products, ingredients, swabs, rinses
8438 Reveal for Peanut Allergen	Total peanut protein	Qualitative: 5 ppm	Swabs, rinses, ingredients
901041L Reveal 3-D Peanut Test	Total peanut protein	Qualitative: low ppm	Swabs and rinses
SESAME ALLERGEN			
902070X BioKits Sesame Assay	Total sesame protein	Quantitative	Products and ingredients
8535 Reveal 3-D Sesame	Total sesame protein	Qualitative	Swabs and rinses
SOY ALLERGEN			
8410 Veratox for Soy	Total soy protein	Quantitative	Processed soy products and ingredients

Neogen test kit and product number	Target ¹	Usage ²	Application ^{3,4}
8411 Alert for Soy	Total soy protein	Qualitative: 5 ppm	Processed soy products, ingredients, swabs, rinses
902093K Reveal 3-D Soy Test	Total soy protein	Qualitative: low ppm	Unprocessed soy protein, swabs and rinses
WALNUT ALLERGEN			
902085J BioKits Walnut Assay	Total walnut protein	Quantitative	Products and ingredients

NOTES: ¹Target refers to the antigen the antibody is detecting; results of test kit are always reported as the food products (i.e., “peanut”, not “peanut protein”). ²Please refer to Appendix A for complete range of quantitation. Refer to Appendix B for limits of determinations. ³Although the Veratox method is primarily used for quantitative analysis, it is possible to use the test as a qualitative screen. Contact Neogen Technical Services for more information. ⁴See page 6 for limitations of ELISA-based food allergen test kits.

APPENDIX A

PRODUCT SPECIFICATIONS FOR VERATOX AND BIOKITS FOOD ALLERGEN TEST KITS

Test kit and product number	Standards prepared from	Antibodies detected	Results reported as	Range of quantitation	Extraction*
8440 Veratox for Almond	A blend of common raw and roasted almonds	Almond proteins	Total almond	2.5–25 ppm (100–1000 ng/mL)	1 to 25 in 10 mM PBS, scoop additive, 15 minutes in a shaker water bath (60°C)
8520 Veratox for Crustacea Allergen	Whole dried shrimp	Crustacea	Total crustacean	2.5–25 ppm	1 to 25 in 10 mM PBS, scoop additive, 30 minutes in shaker water bath (30°C)
8450 Veratox for Egg	Whole dried egg and baked whole dried egg	Unprocessed and heat-processed egg white proteins	Whole dried egg	2.5–25 ppm (100–1000 ng/mL)	1 to 25 in 10 mM PBS, scoop additive, 15 minutes in a shaker water bath (60°C)
8480 Veratox for Gliadin	Wheat gliadin	Prolamins (wheat gliadin, rye secalin, and barley hordein)	Gliadin (gliadin x 2 = gluten)	5–50 ppm (12.5–125 ng/mL)	*1:10 in 40% ethanol, then 1:40 in PBS (1:400)

Test kit and product number	Standards prepared from	Antibodies detected	Results reported as	Range of quantitation	Extraction*
8510 Veratox for Gliadin R5	Wheat gliadin	Prolamins (wheat gliadin, rye secalin, and barley hordein)	Gliadin (gliadin x 2 = gluten)	2.5–40 ppm	**1:10 in 60% ethanol, scoop of additive, then 1:50 in PBS
8420 Veratox for Hazelnut	A mix of raw and roasted hazelnuts	Hazelnut proteins	Total hazelnut	2.5–25 ppm (100–1000 ng/mL)	1 to 25 in 10 mM PBS, scoop additive, 15 minutes in a shaker water bath (60°C)
8500 Veratox for Lupine	Lupine	Lupine protein	Total lupine	2.5–25 ppm (100–1000 ng/mL)	1 to 25 in 10 mM PBS, scoop of additive, 15 minutes in a shaker water bath (60°C)
8470 Veratox for Total Milk	Nonfat dried milk	Caseins and whey proteins	Nonfat dried milk	2.5–25 ppm (100–1000 ng/mL)	1 to 25 in 10 mM PBS, 15 minutes in a shaker water bath (60°C)
902061Y BioKits BLG Assay	Whole dry milk	BLG	BLG	2.5–40 ppm	1 to 10 in extraction solution, dilute 1:10 in diluent
8460 Veratox for Casein Allergen	Nonfat dried milk	Casein	Nonfat dried milk	2.5–15 ppm	1 to 25 in 10 mM PBS, 15 minutes in a shaker water bath (60°C)
8400 Veratox for Mustard	Mustard seed	Mustard protein	Mustard	2.5–25 ppm	1 to 25 in Tris/EDTA buffer, 15 minutes in a shaker water bath (60°C)
8430 Veratox for Peanut	22 varieties of raw and roasted peanuts	Peanut proteins	Total peanut	2.5–25 ppm (100–1000 ng/mL)	1 to 25 in 10 mM PBS, 15 minutes in a shaker water bath (60°C)
902070X BioKits Sesame Assay	Sesame	Sesame protein	Total sesame protein	6.25–100 ppm	1 to 5 in extraction buffer, dilute 1:49 in diluent
8410 Veratox for Soy	Soy protein isolate (SPI)	Soy protein	Soy flour	2.5–25 ppm***	1 to 25 in 10 mM PBS, 15 minutes in a shaker water bath (60°C)

Test kit and product number	Standards prepared from	Antibodies detected	Results reported as	Range of quantitation	Extraction*
902085J BioKits Walnut Assay	Walnuts	Walnut protein	Total walnut protein	2.4–120 ppm	1 to 10 in extraction buffer, dilute 1:1 in diluent

*Additional extraction procedures are required for dark chocolate, cocoa, tannin, and heat-processed samples.

** Additional extraction procedures are required for heat-processed samples.

***October 2012 the Veratox for Soy test kit was changed to report results in ppm soy flour units versus ppm soy protein isolate (SPI).

APPENDIX B

TEST VALIDATION RESULTS FOR VERATOX FOOD ALLERGEN TEST KITS

	Almond	Crustacea	Egg	Gliadin	Gliadin R5	Hazelnut	Lupine
Limit of determination (ppm)	2.5	2.5	2.5	5.0	2.5	2.5	2.5
Reproducibility: Intra-assay variability (%)	3.5	3.0	2.8	4.4	4.3	4.1	4.3
Inter-assay variability (%)	4.1	3.8	3.6	3.2	3.9	3.3	4.1
Linearity: r^2	>0.98	>0.98	>0.98	>0.98	>0.98	>0.98	>0.98
Cross-reactivity	Apricot seed (stone)	No	No	No	No	No	No
Recovery (mean %)	83	86	88	86	110.7	94	79
Stability (months)	11	6	11	6	6	6	6
Assay time (minutes)	30	30	30	30	30	30	30

	Casein	Milk	Mustard	Peanut	Soy
Limit of determination (ppm)	2.5	2.5	2.5	2.5	2.5
Reproducibility: Intra-assay variability (%)	5.0	6.1	4.9	4.3	2.7
Inter-assay variability (%)	6.6	5.2	3.1	4.9	3.4
Linearity: r^2	>0.98	>0.98	>0.98	>0.98	>0.98
Cross-reactivity	No	No	Seeds from the <i>Brassica-ceae</i> family, rapeseed	No	No
Recovery (mean %)	>90	88	88	82	86
Stability (months)	6	7	6	10	9
Assay time (minutes)	30	30	30	30	30

APPENDIX C

TEST VALIDATION RESULTS FOR BIOKITS FOOD ALLERGEN TEST KITS

	BLG	Sesame	Walnut
Limit of determination (ppm)	2.0	1.0	0.25
Reproducibility: Intra-assay variability (%)	<10	2.1	6.31
Inter-assay variability (%)	<10	11.6	7.16
Linearity: r^2	>0.98	>0.98	>0.98
Cross-reactivity	Egg	Poppyseed, linseed	Pecan, quinoa, pistachio, hazelnut, buckwheat
Stability (months)	>9	>6	>9
Assay time (minutes)	120	180	75

APPENDIX D

CONVERTING RESULTS TO PROTEIN FOR VERATOX FOOD ALLERGEN TEST KITS

Overview:

The sensitivity of allergen detection tests is not just a function of the limit of quantitation (LOQ) listed on the test's label, but also is based on the scale the system is calibrated against. It is vital users understand what the results from allergen tests truly represent to ensure the desired sensitivity is achieved.

Various scales of allergenic content have equivalent sensitivities when properly related to one another. Those who prefer to convert their Veratox results from ppm total allergenic food to ppm allergenic protein can do so based on average protein content of these foods, which is included in the table below. Remember, all tests are not created equal. Scale is every bit as important as results in the determination of allergen residues.

Food type	Average protein content	LOQ of Veratox kits using standard extraction on a ppm total allergenic food scale	LOQ of Veratox kits using standard extraction techniques on a ppm protein scale
Milk	35.1%	2.5 ppm nonfat dried milk (NFDM)	0.878 ppm protein
Casein	35.1%	2.5 ppm NFDM	0.878 ppm milk protein, 0.72 ppm casein
Egg	47.35%	2.5 ppm total egg	1.184 ppm protein
Soy	47.01%	2.5 ppm soy flour	1.175 ppm protein
Peanut	25.8%	2.5 ppm total peanut	0.645 ppm protein
Hazelnut	14.95%	2.5 ppm total hazelnut	0.374 ppm protein
Mustard	26.08%	2.5 ppm total mustard	0.652 ppm protein
Almond	21.22%	2.5 ppm total almond	0.531 ppm protein
Gliadin	75.0%	2.5 ppm total gliadin	1.875 ppm protein
Shrimp (crustacea)	22.78%	2.5 ppm total shrimp	0.570 ppm protein
Lupine	15.57%	2.5 ppm total lupine	0.389 ppm protein

SOURCE: United States Department of Agriculture National Nutrient Database Release 24. (Varieties and cultivars can vary in protein content.)

How do I interpret total milk as casein or whey?

If conversion of total milk to casein is desired, one must take into account that NFDM contains 35.1% protein, 80% of which protein is casein. This means a 2.5 ppm Veratox for Total Milk Allergen is 0.702 ppm casein. With whey or β -lactoglobulin (BLG), 20% of the NFDM protein is whey. This means a 2.5 ppm total milk is 0.176 ppm whey.

Target	NFDM level	% protein	% target	Result
NFDM	2.5 ppm	NA	NA	2.5 ppm NFDM
Casein	2.5 ppm	35.1%	80%	0.702 ppm casein
Whey	2.5 ppm	35.1%	20%	0.176 ppm whey

RESOURCES

- **Neogen Europe:** +44 (0) 1292 525 600, www.neogeneurope.com
- **FARRP:** 402/472-4484; www.farrp.org (food allergen consultation, allergen control strategies, confidential lab testing, training videos)
- **Food Standard Agency:** www.food.gov.uk/
- **European Food Safety Authority:** +39 0521 036111, www.efsa.europa.eu/
- **Institute of Food Research:** www.ifr.ac.uk
- **Food Allergy Information:** www.foodallergens.info
- **The Federation of Bakers:** www.bakersfederation.org.uk





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