



Food Allergen Handbook

Testing Guide for Food Allergens

► 1st Edition



Neogen® develops and markets products and services dedicated to food and animal safety. Neogen Food Safety markets diagnostic test kits, culture media, and other products to detect foodborne bacteria, natural toxins, genetic modifications, food allergens, drug residues, plant diseases, and sanitation concerns.

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 that 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.

This United States and Canada edition is based off the original which can be found at www.neogen.com

Food Allergen Handbook

Testing Guide for Food Allergens

What is a Food Allergy?	05
Why test for Food Allergens?	06
Safety around Gluten	07
Regulatory Considerations in USA & Canada	09
How do Rapid Tests Help Prevent Allergen Cross-contact?	11
Screening vs Quantifying Results	12
Neogen Food Allergen Assays	13
How do Neogen's Food Allergen Assays Work?	15
Limitations of ELISA-based Food Allergen Assays	17
Sampling Ingredients, Products, Liquids, and Rinses	19
Environmental Sampling and Extraction	21
Validation and Verification of an Allergen Control Plan	22
Food Allergen Testing FAQs	24
Appendix A: Converting Results to Protein for Neogen Food Allergen Tests	28
Appendix B: Neogen Food Allergen Assays	30
Resources	31



What is a Food Allergy?

The most prevalent allergenic foods include egg, cow's milk (dairy), peanuts, tree nuts, sesame, soy, wheat, fish, and other seafood although researchers have identified more than 160 foods that contain naturally occurring proteins that have been shown to cause allergic reactions.

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 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 numerous 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.

In addition, gluten protein, found in wheat, rye, barley, and related species, has been identified as a major causal agent in several disorders. Celiac disease is the most notable of these disorders. Unlike a wheat allergy, which is a specific immune response to gluten, Celiac disease is a chronic reaction to gluten proteins that results in poor absorption of nutrients in the small intestine due to a consistent inflammatory response.

Why test for Food Allergens?

Testing protects the public

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

Testing protects a company's reputation

Testing provides assurance that allergens that are present in a facility are being controlled to expectations and are compliant with a company's food safety plan.

Testing clean-in-place (CIP) solutions, final product, and equipment after cleaning is finished, can identify sources of cross-contact, and verify cleanliness before the changeover. Testing helps protect against staggering costs and damaged reputation associated with a recall of mislabeled product.

Testing can simplify product labels

Testing can assist with precautionary advisory labeling. Precautionary allergen labeling is a common, voluntary warning to consumers added after the ingredient list.

Its goal is to indicate a product not intended to contain a specific allergen(s) may sporadically contain that allergen due to unintentional and unavoidable cross-contact.

For example, some companies put a precautionary statement such as, “may contain peanut and peanut products” on the ingredient label, even though there may be little chance the product contains any peanut. If testing is done, in conjunction with a thorough risk evaluation, companies may be able to minimize the use of precautionary labels.

Testing improves efficiencies

In companies that use a 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 the product from going to waste or from having to be reworked.

Analysis of a single, or limited, sample is likely not sufficient to ensure product safety. It is important to ensure a rigorous sampling plan is established by your facility prior to conducting any testing. Please refer to the sampling section later in this handbook for proper sampling tips and suggestions.

Safety around Gluten

Wheat is a unique case due to its additional epidemiologic complexity. Gluten is not the only allergenic wheat protein. However, as discussed earlier in this handbook, gluten is the causal agent of Celiac disease. Gluten consists of two classes of proteins (prolamins and glutenins). Gliadin is one of the major prolamin components of gluten.

Other prolamins with similar structures include secalin, found in rye, and hordein, found in barley. Most wheat allergen tests, including Neogen's, are designed for detecting these prolamins. This is why allergen test kit manufacturers frequently market their wheat test kits as "gliadin" or "gluten" test kits. Since gliadin represents approximately 50% of gluten, tests that report results in ppm gliadin can be converted to ppm gluten by multiplying by two.

Those with a wheat allergy or Celiac disease must avoid gluten and rely upon the correct labeling of food to make appropriate, safe food choices. In addition to the wheat allergen implications, food companies voluntarily labeling a product as gluten-free must ensure that their product meets this claim.

The 2008 revision of The Codex Alimentarius Standards 20 ppm (10 ppm gliadin). In 2013, the U.S. Food and Drug Administration (FDA) issued a final ruling defining the term "gluten-free" for food labeling purposes. The rule also defines gluten-free as meaning that the food is either inherently gluten-free or if the gluten content is less than 20 ppm.





Regulatory Considerations in USA & Canada

Different countries have established different lists of priority allergens, based on global and regional research. The FDA has prioritized the Big 9 (milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, soybeans, and sesame) as allergens of highest concern. Health Canada and the European Union Food Information Council (FIC) also include mustard.

FSMA and the food safety plan

As a part of the Food Safety Modernization Act (FSMA), domestic and foreign food facilities that are required to register with section 415 of the Food, Drug, & Cosmetic Act must comply with the requirements for risk-based preventive controls mandated by FSMA as well as the modernized Current Good Manufacturing Practices (CGMPs) of this rule. Finalized in September 2015, the rule requires facilities to have a food safety plan addressing hazard analysis, preventive controls, monitoring, corrective actions, verification, and record-keeping.

As part of this regulation, the establishment of a documented food safety plan is required. This includes a hazard analysis, preventive controls, monitoring, corrective actions, and verification and record keeping procedures. Facilities that manufacture products containing allergens should develop an Allergen Control Plan (ACP) as a part of their food safety plan. An ACP minimizes the risk of sending mislabeled or cross-contaminated products into commerce.

Although the use of food allergen tests is not specifically addressed in legislation, they are valuable tools for assessing and supporting the effectiveness of ACP. In a recent survey of major food manufacturers, the use of food allergen tests has become the “standard of care” in cleaning assessment and validation.

Labeling and reporting allergens

In the United States, the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) requires source allergen labeling in plain English terms when the product contains the priority allergen in the product formulation. In other words, relevant allergens must be labeled in a clearly understood way (e.g. label “milk” instead of “casein”). For most companies, the law requires a comprehensive investigative survey of all their products’ minor ingredients to determine which contain allergens and implement acceptable segregation practices.

Since September 2009, food companies must report within 24 hours any reasonable probability that exposure to a food or raw material could cause serious health consequences or death. Undeclared allergens fall under this requirement. Visit FDA.gov/ReportableFoodRegistry for complete details.



How do Rapid Tests Help Prevent Allergen Cross-Contact?

Diagnostic allergen tests 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 tests 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.



Food Thresholds

Outside of gluten, there are no globally accepted thresholds of any other major allergens that food manufacturers need to meet in order to be considered allergen-free. However, there are some regional exceptions to this. For example, Japan has a regulatory limit of 10 ppm allergenic protein for labeling purposes.

In addition, the Voluntary Incidental Trace Allergen Labelling (VITAL) program, a voluntary allergen risk assessment program for the food industry, has released their third edition of recommended reference doses for several major allergens. With the lack of globally recognized or harmonized reference doses for most major allergens, more companies and regulatory agencies are turning to VITAL 4.0 as a guide for risk assessment and risk management decision making.

The Difference Between Screening and Quantifying Results

Rapid allergen tests come in both screening (qualitative) and quantitative formats, depending on facility needs. Screening tests, such as Neogen's Reveal®/Reveal 3-D product line, are designed to be simple, easy to use, and quick in determining the presence or absence of a target allergen. Quantifying tests, such as Neogen's Veratox® product lines, utilize ELISA technology to determine exact parts per million (ppm) of a target allergen.

A complete table of Neogen's product offerings is available at the end of this handbook.

Neogen is a leader in food allergen rapid diagnostic tests, and offers Neogen's Total Allergen Solution program, which combines rapid screening and quantifying allergen tests, general protein screening tests, and excellent technical support, including ISO-17025 accredited contract lab testing services and hands-on expertise. Please contact a Neogen representative for further information on our Total Allergen Solutions portfolio.



Screening Assays

Reveal® 3-D

The Reveal 3-D product line allows for rapid screening for the presence of low levels of allergen in CIP rinse waters, environmental swabs, and food products with no additional equipment. The 3-D allergen tests utilize an innovative three-line readout: a control line confirms the method has been performed successfully, and two further lines differentiate between no detectable amount, to low contamination and high contamination.



Reveal Multi-Treenut

Reveal Multi-Treenut is designed to detect six tree nut allergens (almond, cashew, hazelnut, pecan, pistachio, and walnut) simultaneously in environmental swabs and CIP rinse waters. The device detects at 5–10 ppm, with results in 10 minutes. Ideal for a quick pre-operation decision and requires minimal hands-on time and equipment.



Quantitative Assays

Veratox®

The Veratox product lines are ELISA tests that determine an exact level of food allergen in about 30 minutes following extraction.





How do Neogen's Food Allergen Assays Work?

Reveal® 3-D/Reveal for Multi Treenut

Neogen's Reveal® 3-D format for the detection of food allergens is a single-step lateral flow immunochromatographic test. The target food allergen is extracted and diluted and wicked onto the device's membrane, where it binds with antibodies specific to that allergen. The test will display results in a three-line format as described below:

- 1. A test line:** Confirming the presence or absence of target allergen.
- 2. An overload line:** This innovative feature will disappear in situations where gross contamination is observed protecting against false-negative results.
- 3. A control line:** Ensuring the user the test is working properly.

Neogen's Reveal for Multi-Treenut test is similar to Reveal 3-D. However, there is no overload line present. This test displays a two-line result, with a test line confirming a positive or negative result, and a control line to ensure the test is working properly.

Veratox®

Neogen's Veratox tests are sandwich enzyme-linked immunoassays (S-ELISAs). The target food allergen protein is extracted from samples and diluted in antibody-coated microwells. After a series of steps, the wells are analyzed on a microwell reader to determine the exact levels of target allergen in units of ppm.

Reveal® 3-D

The Science Inside:
How Reveal® Works



Veratox® VIP ELISA Allergen Kits

The Science Inside:
Allergen Sandwich ELISA



Find out more

For further details on how our Neogen's lateral flow and ELISA assays work, please click or scan the QR codes above or check them out directly on YouTube;

How Reveal works: youtube.com/watch?v=HkSXXUfDAkM

Allergen sandwich ELISA: youtube.com/watch?v=UN7rM6OlipI&t=13s



What are the Limitations of ELISA-based Assays?

ELISA-based food allergen tests are not appropriate for use in certain applications. 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. As mentioned earlier, matrix effect can cause results to vary and it's imperative to test a positive control containing the allergen of interest to determine if the test is fit for purpose. 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, and soy sauce)
- Foods with high levels of tannins and/or phenolics, such as chocolate, cocoa, and wine
- Probiotic cultures
- Enzyme proteases
- Some concentrated food additives, colors, and flavors
- Some oil-based ingredients (e.g., oil, lecithin, oil-soluble flavors, etc.)
- Foods that have undergone certain rigorous processing methods

Also, food allergen tests may report results in ppm based on the total food or based on the amount of protein present. Appendix A lists a chart illustrating how to convert from one to the other, per major allergen.

Neogen test kits generally report results in total food, though please be sure to review the appropriate kit insert for further details, as reporting parameters may vary.



Sampling Ingredients Best Practices

Neogen's experience is that most 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 will increase confidence in subsequent test results.

For further details on proper sampling techniques, please click or scan the QR code below, or check out directly on YouTube.



Sampling variability:
How to take samples
that give the best
results

The following are some general guidelines for ingredient and product sampling.

Please contact Neogen with questions about the adaptability of the guidelines to specific testing needs.

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 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 very fine particle size.
5. Thoroughly mix 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 tests. 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 or blender and utensils between samples.

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 with one of Neogen's food allergen tests, 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.

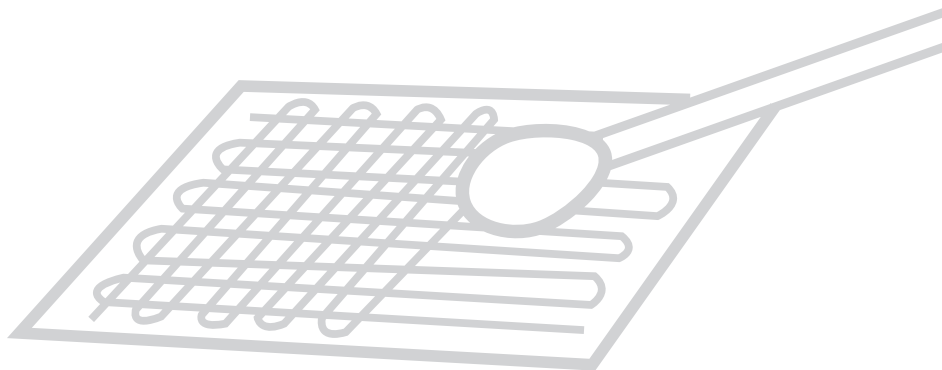


Environmental Sampling and Extraction

Environmental sampling for food allergen detection should be performed after equipment has been thoroughly cleaned, and before production of the following lot has begun. Environmental sampling requires the use of swabs and a specialized extraction of possible allergens from the swabs. 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 tests. 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.

Swabbing should go over a 10 x 10 cm area, using a cross-hatch pattern, as shown in the graphic below. Swabs should be wetted according to kit insert instructions for swabbing dry surfaces. Do not pre-moisten swabs for swabbing wet surfaces.



Validation and Verification of an Allergen Control Plan

The definition of validation as given by Codex Alimentarius “Guidelines for the Validation of Food Safety Control Measures” (2008): “Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.” In this case, the process refers to the cleaning of the facility’s materials after a production run containing allergenic ingredients. The underlying goal for the cleaning process in a food production environment is that it effectively removes all allergenic material to a predetermined safe and satisfactory level. Validation is proof that the goal can be achieved.

It must be based on logical inferences and measurable results, and those results must be translatable to standards that can be utilized for routine monitoring during the normal production cycle. Validation is typically undertaken until the expected outcomes are achieved and then repeated on a scheduled basis or when the underlying assumptions used for validation have changed (such as product formulation, processing change, equipment change, etc.).

Verification, as defined by the Codex Alimentarius, is the application of methods, procedures, tests, and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended. This activity is typically undertaken after each cleaning event, and results are compared against the performance levels obtained during the validation process. While it’s recommended to use specific allergen tests throughout the process, rapid generic protein tests can be used as a part of a verification program. Results that fall outside the validated standard indicate that one or more components of the cleaning process failed. All failures should be confirmed on an allergen-specific test. A facility’s verification process is typically incorporated into its Sanitation Standard Operating Procedure (SSOP). Neogen can assist with implementing, validating, verifying, and maintaining your facility’s individual allergen control plan with our Total Allergen Solutions.

For additional details with using Neogen assays for validation and verification of an ACP, please contact a Neogen sales representative for a copy of the Best Practices for Food Allergen Validation and Verification workbook.

"Obtaining evidence that a control measure...is capable of controlling the hazard to a specified outcome."

Guidelines for the Validation of Food Safety Control Measures
Codex Alimentarius

Food Allergen testing Frequently Asked Questions

Can I test a product on the Reveal® 3-D tests?

All Reveal® 3-D tests are validated for use on environmental swabs and rinses. However, several tests have been validated to measure allergen content on food products and ingredients. For more details, contact a Neogen representative.

What is the difference between “milk” and “total milk”?

“Milk” is a general term for a product that may or may not include both 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 whey can be detected using the same test.

Can I make my quantitative test more sensitive?

The detection limits of Neogen’s quantitative tests have been carefully set based on the most practical levels as determined by the food industry, regulatory, and expert consultants. Methods may be adjusted to provide additional sensitivity. However, this can increase the opportunity for false positive responses due to matrix interferences. Please consult with a Neogen representative for further details.

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 a laboratory environment. Therefore, it is highly recommended that the sample preparation and testing areas, and all instruments, be regularly cleaned.

Furthermore, maintaining consistent lab conditions, including factors such as temperature, to a reasonable standard will reduce potential variance in results.

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

Swabs can be stored for up to 24 hours at 4°C after sample collection, provided they have not been extracted yet. Once extracted, samples from swabs should be evaluated within four hours. All other sample extracts should also be tested within four hours.

Where are the best locations to sample the environment with testing 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 buildup of food residues.

When should you test CIP rinse solutions?

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

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.

What are the limitations of general protein testing in food allergen monitoring?

General protein tests do not target specific allergen proteins and are not appropriate for developing, implementing, and validating ACPs. Companies should be using specific allergen tests for implementing and validating an ACP. It is recommended that allergen tests are used for verification whenever possible, but general protein testing (such as Neogen's AccuClean® Advanced) can be an effective tool as a part of verification for an ACP. They can be used for spot verification to determine that validated ACPs and SSOPs remain effective. Allergen tests should be used to confirm any positive results from a protein test. Furthermore, any changes to a facility's allergen control plan due to a different product or process should be validated with allergen tests.

How does heat processing affect the recovery of food allergens on the Neogen tests?

Neogen has carefully designed its tests to detect allergenic protein across a wide variety of food products, both processed and unprocessed. In rare cases, highly refined proteins may not be detectable. Neogen's technical experts can assist with suggesting products that fit best for your facility.

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.

Are there confirmatory analytical methods for allergen testing?

Currently, no confirmatory methods beyond ELISA exist. However, for confirmation of test results, there are many third-party laboratories that run full, quantitative ELISA methods. Although instrumental methods such as PCR and LC/MS/MS do exist, they are not currently recognized beyond the scope of research purposes only.

Why are the Reveal® 3-D tests set at 5 ppm?

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

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

Limit of detection (LOD) refers to the lowest concentration where the test picks up a signal that can be differentiated from a blank sample and not due to background noise. This is usually well below the standard curve and is not considered a valid result. A LOD is determined as the mean of ten evaluations of a known negative sample, plus two standard deviations. A limit of quantitation (LOQ) is the lowest point where the result is quantifiable by the test on a standard curve. Most quantitative products identify this as the first non-zero control in that test. Results below LOQ but above LOD are generally considered not quantitatively valid, but may have validity from a qualitative standpoint. However, it is critical to ensure that apparent qualitative positives are not due to background noise from the sample matrix being analyzed.

How many ppm can be detected from a rinse?

In general, the sensitivity for the 3-D tests for rinses ranges from 5–10 ppm; however, this can fluctuate based on the type and concentration of cleaning chemicals and sanitisers 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. Please refer to the individual product validation report for further information.

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 the sample size widely fluctuates depending on the amount of material captured on the surface of the swab, a ppm definition cannot apply. Instead, it is appropriate to define sensitivity in terms of μg of contaminants found in a 100 cm^2 surface (10 $\text{cm} \times 10 \text{ cm}$ surface was used during validation). Given this definition, 5–20 μg of allergen per 100 cm^2 is achievable; however, this can vary depending on the surface and type and concentration of cleaning chemicals or sanitizers present on the surface.

How many ppm can be detected from a food sample?

In general, the sensitivity for the 3-D tests that have food testing capability range between 5–10 ppm. However, this can fluctuate depending on matrix being tested. Neogen encourages facilities to validate their matrix on the respective 3-D kit needed. For further information, please reference the individual product validation report or contact a Neogen representative.

Converting results to protein for Neogen® Food Allergen assays

Overview:

The sensitivity of allergen detection tests is not just a function of the sensitivity 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 results from ppm total allergenic food to ppm allergenic protein can do so base on the average protein content of these foods, which is included in the table below. Remember — all tests are not created equal. The scale is as important as results in the determination of allergen residues.

Food Type	Average Protein Content*	LOQ of Veratox® Kit on a ppm Total Allergenic Food Scale	LOQ of Veratox Kit on a ppm Protein Scale	Detection Limit of Reveal®/Reveal 3-D on a ppm Total Allergenic Food Scale	Detection Limit of Reveal/Reveal 3-D on a ppm Protein Scale
Almond	21%	2.5 ppm almond	0.53 ppm protein	5 ppm almond	1.1 ppm protein
Casein	36%	2.5 ppm NFDM	0.91 ppm protein	N/A	N/A
Cashew	18%	1 ppm cashew (VIP)	0.2 ppm protein	5 ppm (multi-treenut)	1 ppm protein
Crustacean	20%	2.5 ppm shrimp	0.50 ppm protein	5 ppm shrimp	1 ppm protein
Egg	48%	2.5 ppm egg	1.20 ppm protein	5 ppm egg	2.4 ppm protein
Hazelnut	15%	2.5 ppm hazelnut	0.37 ppm protein	5 ppm hazelnut	0.7 ppm protein
Milk	35%	2.5 ppm NFDM	0.91 ppm protein	5 ppm NFDM	1.8 ppm protein
Mustard	26%	2.5 ppm mustard	0.65 ppm protein	5 ppm mustard	1.3 ppm protein
Peanut	26%	2.5 ppm peanut 1 ppm peanut (VIP)	0.65 ppm protein 0.25 ppm protein (VIP)	5 ppm peanut	1.3 ppm protein

Food Type	Average Protein Content*	LOQ of Veratox® Kit on a ppm Total Allergenic Food Scale	LOQ of Veratox Kit on a ppm Protein Scale	Detection Limit of Reveal®/Reveal 3-D on a ppm Total Allergenic Food Scale	Detection Limit of Reveal/Reveal 3-D on a ppm Protein Scale
Pecan	11%	N/A	N/A	10 ppm (multi-treenut)	1 ppm protein
Pistachio	20%	N/A	N/A	10 ppm (multi-treenut)	2 ppm protein
Sesame	17%	2.5 ppm sesame	0.42 ppm protein	5 ppm sesame	0.8 ppm protein
Soy	51%	2.5 ppm soy flour	1.29 ppm protein	5 ppm soy flour	2.6 ppm protein
Walnut	15%	0.15 ppm	0.40 ppm protein	1 ppm (multi-treenut)	0.2 ppm protein

*Protein content based on United States Department of Agriculture National Nutrient Database Release 28. Varieties and cultivars can vary in protein content. Assays that detect coconut and gliadin/gluten are not included here since, by design, they only report in a ppm protein scale.

How do I interpret total milk as casein or whey?

If the conversion of total milk to casein is desired, remember that Neogen’s total milk kits are based on a non-fat dried milk (NFDM) scale. NFDM contains 35% protein, 80% of which is casein. This means a 2.5 ppm Veratox® for Total Milk Allergen is 0.7 ppm casein. With whey or β-lactoglobulin (BLG), 20% of the NFDM protein is whey. This means a 2.5 ppm total milk is 0.2 ppm whey.

Target	NFDM Level	Protein %	Target %	Result
NFDM	2.5 ppm	NA	NA	2.5 ppm NFDM
Casein	2.5 ppm	35%	80%	0.7 ppm casein
Whey	2.5 ppm	35%	20%	0.2 ppm whey

Why is soy unique?

Soy can be processed in various ways, each affecting functionality and protein concentration. The adulterating material must be known to convert to protein accurately. Generally, the following protein levels apply:

- Soy flour > 50% protein
- Soy protein concentrate > 65% protein
- Soy protein isolate > 90% protein

Neogen® Food Allergen Diagnostic Testing Products

Qualitative (Screening) Assays		Quantitative Assays	
Product	Item #	Product	Item #
Reveal® for Multi Treenut	8555	Veratox for Almond	8440
Reveal 3-D for Almond	902086G	Veratox for Casein	8460
Reveal 3-D for Coconut	8565	Veratox for Crustacea	8520
Reveal 3-D for Crustacea	902081S	Veratox for Egg	8450
Reveal 3-D for Egg	8450	Veratox for Gliadin	8480
Reveal 3-D for Gluten	8505	Veratox for Gliadin R5	8510
Reveal 3-D for Hazelnut	902087E	Veratox for Hazelnut	8420
Reveal 3-D for Total Milk	8479	Veratox for Total Milk	8470
Reveal 3-D for Mustard	8405	Veratox for Mustard	8400
Reveal 3-D for Peanut	901041L	Veratox for Peanut	8430
Reveal 3-D for Sesame	8535	Veratox for Sesame	8530
Reveal 3-D for Soy	902093K	Veratox for Soy	8410
		Veratox VIP Peanut	8430M
		Veratox VIP Cashew	8570
		Veratox VIP Walnut	8550

Neogen® Corporation, 517.372.9200; info.neogen.com/USAC-FoodSafety
Test kits, confidential allergen lab testing

- **FARRP**, 402.472.4484; www.farrp.org
Food allergen consultation, allergen control strategies, confidential lab testing, training videos

- **FDA**, fda.gov

- **WHO-FAO Codex Alimentarius International Food Standards**,
fao.org/fao-who-codexalimentarius

- **Food Allergy Research and Education (FARE)**, foodallergy.org

- **Consumer Brands Association**, consumerbrandsassociation.org

- **Health Canada**, 613.957.2991; hc-sc.gc.ca

- **Association for Dressings and Sauces**, dressings-sauces.org

- **Institute of Food Technologists**, IFT.org

- **American Institute of Baking (AIB)**, 800.633.5137; AIBonline.org

- **VITAL Program-Allergen Bureau**, allergenbureau.net/vital



We are here to support you

Neogen Offers Global Support for Allergen Detection



Method Trial

- Technical sales support
- Education materials
(by segment, competitive tech, assay type)
- Demonstration or initial evaluation

Method Application

- Matrix Qualification Program*
- Studies and publications
- Integration into lab workflow
- Training

Routine Use

- Product availability
- Results interpretation
- Technical concerns

* Contact your local Neogen representative for more information.

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