# AlerTox® ELISA Peanut Kit

**For the quantitative detection of peanut proteins in food products**

REF KIT3048 (96 reactions)

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# 1. Introduction

Peanut allergies are the most common food allergy in children and the third most common food allergy in adults. Children are less likely to outgrow peanut allergies than other food allergies, and some people have adult-onset peanut allergies. The prevalence varies by region, affecting approximately 2% of the general population in Western countries.

Peanuts are legumes (i.e., not tree nuts), but approximately 20 – 30% of people who have peanut allergies are also allergic to one or more tree nuts. There can also be cross-reactivity with allergenic proteins from another legume (i.e., lupine), so there is a subset of patients who are allergic to both peanuts and lupines.

Peanuts contain approximately 23% protein. There are at least 12 allergenic peanut proteins, some of which are heat resistant, making them stable to various production processes. Because peanut allergies are one of the most common causes of fatal anaphylaxis, they are considered one of the "Big 9" allergens requiring accurate and reliable detection for consumer safety and compliance with food labeling regulations.

In addition to the AlerTox® ELISA Peanut Kit, some manufacturers may want to use AlerTox ELISA Kits that detect other legumes, such as lupine and soy. AlerTox ELISA Kits are also available for tree nuts, including almonds, cashews, hazelnuts, macadamias, pistachios and walnuts. The same sample preparation can be used with the AlerTox ELISA tests mentioned above.

**Note**: Read this manual carefully before starting the test. The test must be performed by thoroughly trained staff.

## 1.1 Test Sensitivity and Specificity

The AlerTox ELISA Peanut Kit detects and quantifies peanut proteins in foods, such as cakes, chocolate, desserts, pastries, pralines, puddings, yogurt and other foodstuffs, which may be raw, heated or baked. The limit of detection (LOD) is 0.3 ppm (mg of whole peanut per kg of sample), the limit of quantification (LOQ) is 1.0 ppm whole peanut (mg/kg) and the detection is quantitative between 1.0 and 30 ppm whole peanut (see *Section 6.2.1, Summary of Specifications*, for more details). See *Section 4, Results Calculations*, for more details about the expression of the results.

The cross-reactivity with other food matrices is shown in the following table:

| Cross-Reactive Matrix | Percent Cross-Reactivity (%) |
|---|---|
| Cayenne | 0.0012 |
| Gum arabic | 0.0006 |
| Cocoa | 0.0002 |
| Clove | 0.0001 |

**Note**: Almond, carob gum, fenugreek, lentil, pecan and soy flour showed results between 0.5 LOQ and 1 LOQ and may provide values above the LOQ.

See *Section 6.2.2, Recovery* and *Section 6.2.3, Non-Cross Reactivity*, for additional data.

**Important**: Do not modify the protocol with respect to the timing, temperatures, plate washing, pipetting volumes, types of buffers or pH values of the buffers. Any of these protocol modifications will invalidate the test system.

## 1.2 Sample Preparation

The AlerTox ELISA Peanut Kit is one in a series of twenty related allergen test kits from Hygiena®. Sixteen different allergens, including peanut, can be detected and measured using a single sample extract with these different allergen-specific ELISA tests, while a few need individual extractions. See *Section 6.1, Sample Extraction Compatibility,* for more details.

### 1.3 Test Principle

The AlerTox ELISA Peanut Kit works on the principle of a quantitative sandwich ELISA. The antigen concentration is directly proportional to the color intensity of the test sample. Here is a brief overview of the sandwich ELISA test:

1. Primary antibodies directed against peanut proteins are bound on the surface of a microtiter plate. Peanut-containing standards or test samples are placed into the wells of the microtiter plate. After a 20-minute incubation at room temperature (15 to 25 °C, 59 to 77 °F), the wells are washed with washing solution to remove unbound material.
2. Peroxidase-conjugated secondary antibodies directed against peanut proteins are put into the wells, and after a second 20-minute incubation, the plate is washed again.
3. The Substrate Solution is added, and the plate is incubated for another 20 minutes, resulting in the development of a blue color in positive wells. The addition of the Stop Solution inhibits further color development, and the color turns yellow. The yellow color is measured photometrically at 450 nm ($OD_{450\,nm}$).

## 2. Materials and Storage

### 2.1 Materials Supplied in the Kit

| Item | Description | 96 wells |
|---|---|---|
| 1 | Breakable strips of 8 wells, each coated with anti-peanut primary antibodies. In a re-sealable foil bag containing a frame and drying agent. Ready to use. | 12 strips |
| 2 | 5 AlerTox Peanut Standards, concentrations: 0 – 1 – 5 – 15 – 30 ppm. Ready to use. | 5 x 3 mL |
| 3 | Conjugate: Peroxidase-conjugated, anti-peanut secondary antibodies. Ready to use. | 1 x 15 mL |
| 4 | Substrate Solution, containing trimethylbenzene (TMB). Ready to use. | 1 x 15 mL |
| 5 | Stop Solution, containing sulfuric acid ($H_2SO_4$). Ready to use. | 1 x 15 mL |
| 6 | 10X Extraction & Sample Dilution Buffer. | 4 x 30 mL |
| 7 | 10X Washing Solution. | 2 x 60 mL |

### 2.2 Storage Conditions and Stability

- All kit components should be kept at 2 to 8 °C (36 to 46 °F) in the dark. DO NOT FREEZE.
- Return all reagents to 2 to 8 °C (36 to 46 °F) immediately after use.
- The diluted Washing Solution (1X) can be used for 4 weeks when stored at 2 to 8 °C (36 to 46 °F).

  **Important**: If needed, redissolve precipitants by warming the 10X Washing Solution at 37 °C (99 °F) for 15 minutes before dilution. Do not use the buffer if the precipitant does not redissolve.
- The diluted Extraction & Sample Dilution Buffer (1X) can be used for 1 week when stored at 2 to 8 °C (36 to 46 °F).

  **Important**: If needed, redissolve precipitants by warming the 10X Extraction & Sample Dilution Buffer at 37 °C (99 °F) for 15 minutes before dilution. Do not use the buffer if the precipitant does not redissolve.
- The Sample Extracts are stable for at least 24 hours at 2 to 8 °C (36 to 46 °F) or longer when frozen.

### 2.3 Material Required but Not Provided

- AlerTox Polyphenol Additive (Product No. ASY3213), only for samples with polyphenols and antioxidants*
- Multi-channel pipettor: 50 – 200 µL
- Sterile pipette tips
- Pipettors: 10 – 100 µL, 100 – 1,000 µL
- Water bath, adjustable to 60 °C (140 °F)
- 15 – 30 mL containers for the extractions
- ELISA Plate Reader with filter (450 nm) (Absorbance 96 ELISA Reader, Product No. MCH3005, or similar)
- Centrifuge
- Distilled water
- Stomacher, Mill, Mortar, Blender, etc.
- Vortex mixer

* Examples of foods rich in polyphenols, including tannins, and antioxidants are chocolate, tea, coffee, wine, purple corn and corn fiber, soy, berries and legumes, such as chickpeas or lentils.

### 2.4 Optional Materials/Equipment

- Homogenizer for sample extraction
- Repeating pipettor to minimize assay drift
- *Recommended*: An ELISA plate washer system to reduce the washing time and improve consistency

AlerTox ELISA Kits have been validated on fully automated ELISA systems (such as the BEAR Automated ELISA Robot). For validation details, contact us at www.hygiena.com/support.

## 3. Test Procedure

### 3.1 Reagent Preparation

We advise preparing reagents immediately before use and only preparing the amount necessary for the number of samples plus the 5 standards. Duplicate measurements of each sample and standard are recommended based on good laboratory practices (GLP) and quality control requirements.

**Important**: All reagents must be at room temperature (15 to 25 °C, 59 to 77 °F) at the time of use.

#### *3.1.1 Extraction & Sample Dilution Buffer*

Dilute the 10X Extraction & Sample Dilution Buffer 1:10 with distilled water to create the 1X solution.

**Important**: If needed, redissolve precipitants by warming the 10X Extraction & Sample Dilution Buffer at 37 °C (99 °F) for 15 minutes before dilution. Do not use the buffer if the precipitant does not redissolve.

**Note**: You will need the following amounts for each sample in your test:

| Sample Type | Amount of Sample | Amount of 1X Extraction & Sample Dilution Buffer |
|---|---|---|
| Solid | 0.5 g | 10 mL |
| Liquid | 0.5 mL | 9.5 mL |

#### *3.1.2 Washing Solution*

Dilute the 10X Washing Solution 1:10 with distilled water to create the 1X solution.

**Important**: If needed, redissolve precipitants by warming the 10X Washing Solution at 37 °C (99 °F) for 15 minutes before dilution. Do not use the buffer if the precipitant does not redissolve.

**Note**: You will need approximately 2.5 mL of 1X Washing Solution per well.

#### *3.1.3 ELISA Plate*

To prepare the ELISA plate, open the foil bag, remove the number of strips required to run the tests (samples plus the 5 standards, all in duplicate) and put the strips into a frame.

**Notes**:

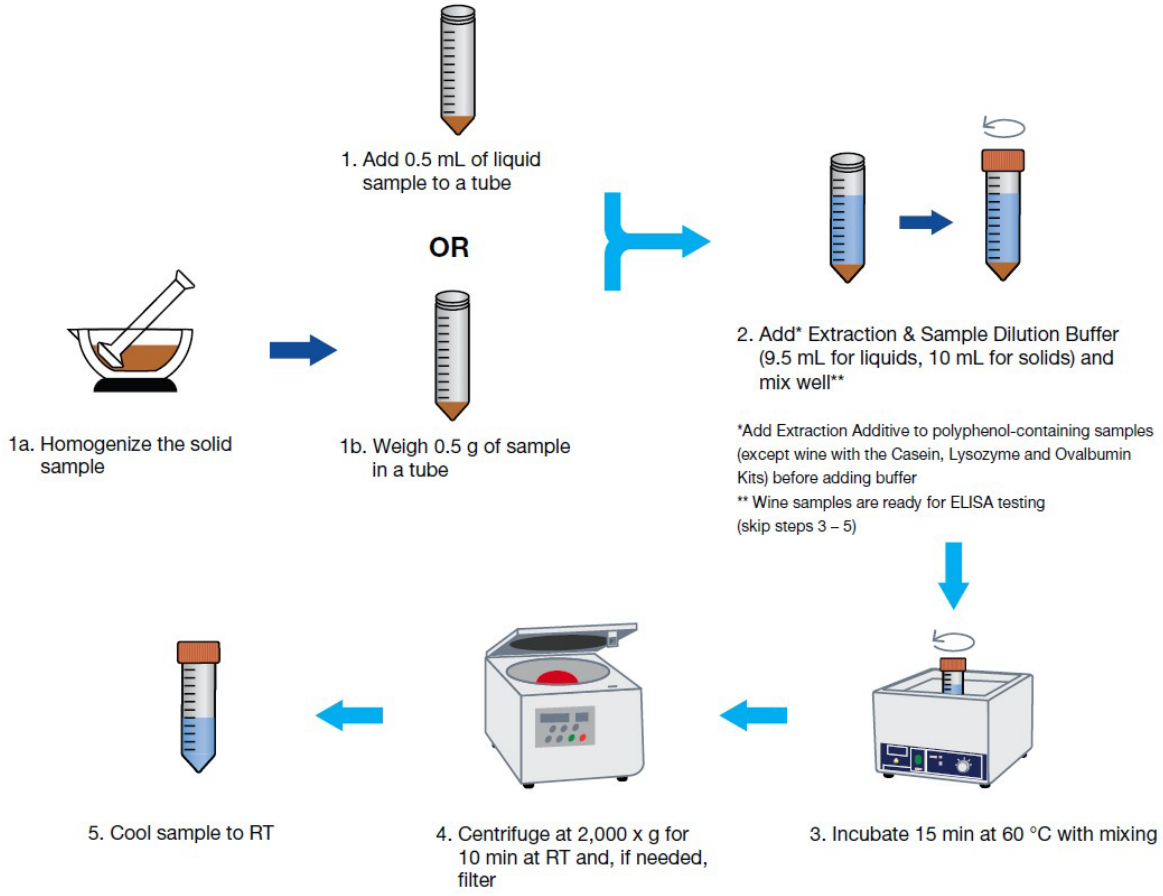
- When opening the foil bag for the first time, be careful not to cut the ziplock off the bag.
- Unused wells must be stored in the foil bag with the drying agent at 2 to 8 °C (36 to 46 °F). Ensure the ziplock on the foil bag is sealed tightly.

### 3.2 Sample Preparation

**Important**: See *Appendix A* for the sample preparation protocol for samples containing polyphenols, tannins or antioxidants. For other samples, follow the procedure below:

1. Resuspend sample in 1X Extraction & Sample Dilution Buffer based on sample type:
    a. For solid samples:
        i. Maximize the homogeneity of the sample by finely pulverizing a minimum of 5 g of sample in a mortar, impact mill or a similar device.
        ii. Resuspend 0.5 g of the homogenized mixture in 10 mL of 1X Extraction & Sample Dilution Buffer.
    b. For liquid samples:
        i. Add 0.5 mL of the liquid sample to 9.5 mL of 1X Extraction & Sample Dilution Buffer.
2. Mix well.
3. Incubate the mixture for 15 minutes in a preheated water bath at 60 °C (140 °F), shaking samples every 2 minutes to ensure homogeneity.
4. Centrifuge the mixture for 10 minutes at 2,000 x g at room temperature (15 to 25 °C, 59 to 77 °F). If the supernatant is still not completely separated from the precipitate, filter the supernatant.
5. Cool the sample extract (supernatant or filtrate) to room temperature (15 to 25 °C, 59 to 77 °F).

### *3.2.1 Workflow Overview*




**Important:** See special instructions for sample extraction for the AlerTox ELISA Casein, Crustacean, Fish, Histamine, Lysozyme and Milk Kits.

## 3.3 ELISA Procedure

**Important**: The most critical points of the ELISA procedure are the temperature, timing and plate washing. Insufficient washing will result in poor precision and false results.

**Note**: For higher reproducibility, we recommend using a well-maintained, automated plate washer in steps 3 and 6 below.

1. Add 100 µL of the standards or sample extracts in duplicate into the appropriate wells of the microtiter plate.

   **Note**: See *Section 7, Example Assay Layout*. If you have a large number of samples, pipette one set of standards before the samples and the duplicate set of standards after the samples and use the arithmetic mean values for calculations.

2. Incubate for 20 minutes at room temperature (15 to 25 °C, 59 to 77 °F).

   **Important**: Do not shake the plate during this incubation.

3. Wash plates **three (3)** times with 300 µL of 1X Washing Solution per well.

   **Note**: At the end of the automated washing or between each manual wash, invert the plates and strike them against clean, dry paper towels to empty the wells and remove residual liquid.

4. Add 100 µL of Conjugate Solution into each well.
5. Incubate for 20 minutes at room temperature (15 to 25 °C, 59 to 77 °F).

   **Important**: Do not shake the plate during this incubation.
6. Wash plates **five (5)** times with 300 µL of 1X Washing Solution per well.

   **Note**: At the end of the automated washing or between each manual wash, invert the plates and strike them against clean, dry paper towels to empty the wells and remove residual liquid.
7. Pipette 100 µL of Substrate Solution into each well.
8. Allow the reaction to develop in the dark (the substrate is light-sensitive) for 20 minutes at room temperature (15 to 25 °C, 59 to 77 °F).

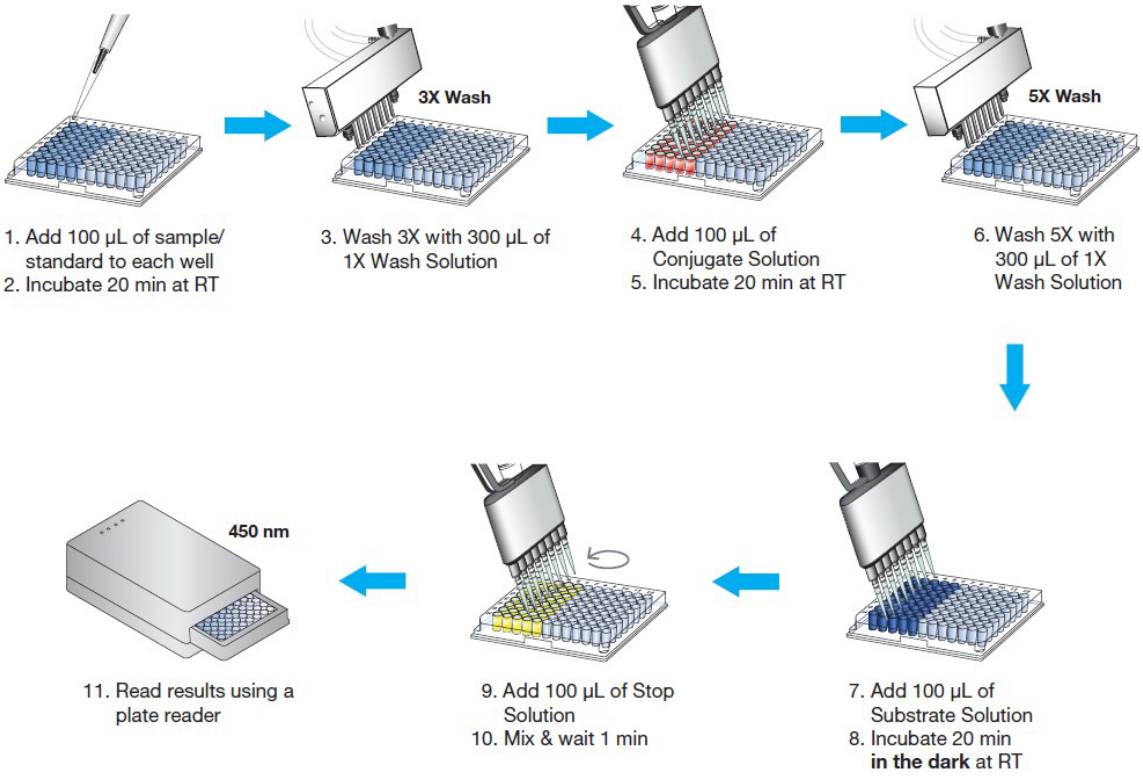
   **Important**: Do not shake the plate during this incubation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution (0.5 M $H_2SO_4$) into each well.
10. Gently shake the plate by hand and wait for 1 minute.

    **Note**: Wells containing blue color turn yellow in the presence of peanut proteins.
11. To measure results, use an ELISA plate reader with a 450 nm filter ($OD_{450\ nm}$), following the instrument manufacturer's instructions.

    **Note**: Measure the color change within 30 minutes.

    **Important**: If any sample results fall outside the range of the peanut standard curve, do not extrapolate the data. Instead, dilute the sample extract further with 1X Extraction & Sample Dilution Buffer and repeat the ELISA test using this diluted sample extract and standards, in duplicate.

### *3.3.1 Workflow Overview*



## 4. Results Calculations

**The results are measured as the concentration of whole peanut and not peanut protein.** See Step 5 below for a conversion factor to calculate peanut protein concentrations.

The standards are prepared for a direct determination of whole peanut concentrations in samples. The dilution of samples in the extraction process, as described in the sample preparation procedures, is already taken into consideration when calculating levels. However, results must account for any additional dilution (e.g., due to high sample concentration or some alternative sample extraction procedures) (Step 4, notes below). Use the *AlerTox ELISA Calculation Worksheet* (available at www.hygiena.com/documents) or the following instructions to calculate results.

**Important**: Do not use the *AlerTox ELISA Calculation Worksheet* if the Zero Standard on the plate reader software is defined as the Blank for the calculation of $B - B_0$.

When interpreting the results, the arithmetic mean is used for calculations.

1. Calculate the mean OD value ($OD_{450\,nm}$) for each set of duplicate reference standards and duplicate samples.
2. Subtract the mean value of the Zero Standard from each mean OD value of standards or samples ($OD - OD_{Standard\ 0} = B - B_0$). See below, *Example Assay Data*.

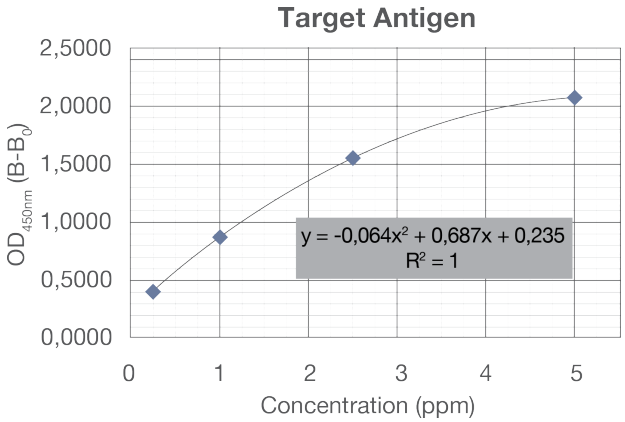
   **Important:** If the Zero Standard on the plate reader software is defined as the Blank for the calculation of $B - B_0$, skip this step.
3. To create the standard curve, plot the adjusted OD values of standards 1 to 4 on the y-axis versus the concentration of whole peanut in ppm on the x-axis. See below*, Example of a Typical Standard Curve*.
4. For each sample extract, find the value $B - B_0$ on the y-axis. Then, read the corresponding value on the x-axis of the standard curve to determine the concentration of whole peanut.

   **Note**: When using the standard sample preparation procedure (Section 3.2), it is not necessary to multiply the resulting concentration of the foodstuff sample by the dilution factor of 20.
5. To convert ppm of whole peanut to ppm of peanut protein, divide the results by 4.3 [1].

**Example Assay Data**

| Standard | Target Antigen [ppm] | Mean $OD_{450nm}$ | $B - B_0$ |
|---|---|---|---|
| Zero | 0.0 | 0.108 | — |
| 1 | 2.0 | 0.265 | 0.157 |
| 2 | 10.0 | 0.606 | 0.498 |
| 3 | 25.0 | 1.193 | 1.085 |
| 4 | 50.0 | 1.928 | 1.820 |

**Example of a Typical Standard Curve**




## 5. General Precautions

- If your skin comes in contact with toxic or irritating substances, rinse the affected area with plenty of water and seek medical attention if needed. Please refer to the SDS, available at www.hygiena.com/SDS.
  - The Substrate Solution contains TMB, which is highly toxic if inhaled, ingested or contacts skin. Please refer to the SDS.
  - The Stop Solution contains $H_2SO_4$, which is corrosive. Please refer to the SDS.

- Handle the test kit in accordance with GLP.
  - Do not use reagents beyond the expiration date of the kit.
  - Handle all solutions with gloves.
  - During the sample extraction, avoid cross-contamination.
  - Devices, such as a blender, must be cleaned after each sample preparation.
  - Use sterile pipette tips.
  - Do not exchange reagent vial caps.
  - Do not interchange reagents between kits of different lot numbers.
- Do not alter reagents. Doing so can cause inaccurate results.
- All reagents must be equilibrated to room temperature (15 to 25 °C, 59 to 77 °F) before use.
- Do not use solutions if they become cloudy or precipitate. The only exceptions are 10X Washing Solution and 10X Extraction and Sample Dilution Buffer, which may have crystalline precipitants that must be completely dissolved before use (see Section 2.2).
- Substrate Solution is light sensitive. Avoid exposure to direct light and store in the dark.
- Use only distilled water for the dilution of concentrated buffers.
- Do not allow wells to dry completely.
- Avoid incubating microtiter plates on cold work benches.

## 6. Additional Information

### 6.1 Sample Extraction Compatibility

The following AlerTox ELISA kits share the same sample preparation protocol, meaning the sample extract can be tested using 16 different ELISA Assays:

| Compatible Sample Extractions | | | |
|---|---|---|---|
| AlerTox ELISA Almond | AlerTox ELISA BLG* | AlerTox ELISA Cashew | AlerTox ELISA Coconut |
| AlerTox ELISA Egg | AlerTox ELISA Hazelnut | AlerTox ELISA Lupine | AlerTox ELISA Lysozyme† |
| AlerTox ELISA Macadamia | AlerTox ELISA Mustard | AlerTox ELISA Ovalbumin | AlerTox ELISA Peanut |
| AlerTox ELISA Pistachio | AlerTox ELISA Sesame | AlerTox ELISA Soy (STI) | AlerTox ELISA Walnut |

* BLG = β-lactoglobulin

† Only the wine extract is compatible. (Cheese and other food extracts are not compatible.)

Individual samples must be extracted separately when using the following kits:

| Individual Sample Extractions Required | | |
|---|---|---|
| AlerTox ELISA Casein | AlerTox ELISA Crustacean | AlerTox ELISA Fish |
| AlerTox ELISA Histamine* | AlerTox ELISA Lysozyme† | AlerTox ELISA Milk |

* The AlerTox ELISA Histamine Kit is based on a competitive ELISA test, while all other AlerTox ELISA Kits are based on sandwich ELISA tests.

† Cheese and other food samples, except for wine, must be extracted separately.

## 6.2 AlerTox ELISA Peanut Kit

### *6.2.1 Summary of Specifications*

| Specification | AlerTox ELISA Peanut* |
|---|---|
| Results | Concentration of whole peanut |
| Limit of Detection (LOD) | 0.3 ppm |
| Limit of Quantification (LOQ) | 1.0 ppm |
| Standard Range | 0.0 – 30 ppm |
| Quantification Range | 1.0 – 30 ppm |
| Calculation Factor† | Peanut protein [1] Divide by 4.3 |

* ppm = mg of whole peanut per kg of sample

† Use the calculation factor to convert the results to the concentration of peanut protein.

For lot-specific assay data and acceptance/rejection criteria for measured values, see the Certificate of Analysis (www.hygiena.com/COA).

### *6.2.2 Recovery*

| Matrix* | Recovery (%) |
|---|---|
| Chocolate | 96 |
| Cookies | 97 |
| Cornflakes | 102 |
| Hazelnut | 93 |
| Ice cream | 93 |

* Tested in typical matrices.

### *6.2.3 Non-Cross Reactivity*

Of the matrices that were tested, the following were found to be non-cross-reactive with the AlerTox ELISA Peanut Kit:

| Non-Cross-Reactive Matrices | | | | |
|---|---|---|---|---|
| Adzuki bean | Almond* | Apple | Apricot | Barley |
| Bean, white | Beef (cooked) | Beef (raw) | Brazil nut | Buckwheat |
| Cabbage, white | Caraway | Cardamom | Carob gum* | Carrot |
| Cashew | Celery | Cherry | Chestnut | Chia |
| Chicken | Chickpea | Chili | Cinnamon | Coconut |
| Cod | Corn | Cumin | Dill | Duck |
| Egg | Fennel | Fenugreek* | Flaxseed | Garden cress |
| Garlic (fresh) | Garlic (granulated) | Gelatin, cow | Ginger (ground) | Ginger (fresh) |
| Gliadin | Guar gum | Hazelnut | Horseradish | Kidney bean |
| Kiwi | Lamb | Leek | Lentil* | Lupine |
| Macadamia | Milk, cow | Milk, cow (powder) | Milk, goat | Mustard |
| Nutmeg | Oats | Onion | Paprika | Pea |
| Peach | Pecan* | Pepper, black | Pine seed | Pistachio |
| Poppy | Pork | Potato | Prawn (cooked) | Prawn (raw) |
| Pumpkin seed | Radish | Rapeseed | Rice | Rye |
| Saccharose | Sesame | Shrimps | Soy flour* | Soy lecithin |
| Split pea | Sunflower seed | Thyme | Tofu | Tomato |
| Turkey | Turmeric | Walnut | Wheat | |

***** Almond, carob gum, fenugreek, lentil, pecan and soy flour showed results between 0.5 LOQ and 1 LOQ and may provide values above the LOQ.

## 7. Example Assay Layout

**S0:** Zero Standard (without antigen): the mean value = $B_0$.

**S1 – S4:** Standards: the mean value = B.

**SP:** Samples: the mean value = B.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | **S0** | **S0** | SP4 | SP4 | SP12 | SP12 | | | | | | |
| B | **S1** | **S1** | SP5 | SP5 | Etc. | Etc. | | | | | | |
| C | **S2** | **S2** | SP6 | SP6 | Etc. | Etc. | | | | | | |
| D | **S3** | **S3** | SP7 | SP7 | Etc. | Etc. | | | | | | |
| E | **S4** | **S4** | SP8 | SP8 | Etc. | Etc. | | | | | | |
| F | SP1 | SP1 | SP9 | SP9 | Etc. | Etc. | | | | | | |
| G | SP2 | SP2 | SP10 | SP10 | Etc. | Etc. | | | | | | |
| H | SP3 | SP3 | SP11 | SP11 | Etc. | Etc. | | | | | | |

## 8. References

1. USDA, US Department of Agriculture (2023) Peanuts, raw. https://fdc.nal.usda.gov/fdc-app.html#/food-details/2515376/nutrients.

## 9. Disclaimer

Field of use: Use the Hygiena product for research and development, quality assurance and quality control under supervision of technically qualified persons. The information generated from the Hygiena product is only to be used in conjunction with the user's regular quality assurance program. The Hygiena product should not be used as the sole basis for assessing the safety of products for release to consumers. Data obtained from the Hygiena product must not be used for human diagnostic or human treatment purposes. Before using product, read the *Limitation of Warranty and Liability* (available in the *Hygiena General Terms and Conditions* at www.hygiena.com/terms-and-conditions).

These products are made from high-quality raw materials. No warranty of any kind is made, either expressed or implied, as to their suitability other than to measure the target antigen content when used exactly in accordance with these instructions, except regarding the quality of these materials.

Use of the kit for any other purpose is outside its intended use. For matrices that have not been previously validated, Hygiena cannot guarantee that the kit is fit for purpose and that the results obtained for these matrices are accurate. Customers may choose to use the product on unvalidated food or surface matrices; however, Hygiena strongly recommends that users perform their own fit-for-use testing to confirm suitability and performance in their specific application. Any damages, including consequential or special damage or expense arising directly or indirectly from using this product, are limited to the replacement value of the kit.

For additional information or assistance with matrix validation, contact Hygiena at www.hygiena.com/support. All Hygiena Terms and Conditions apply and can be found at: www.hygiena.com/terms-and-conditions.

## 10. Contact Information

For more information, visit www.hygiena.com/contact. For technical support, visit www.hygiena.com/support.

## 11. Change Index

INS3022 REVD, July 2020
*Clarified parts of the conversion factors table.*

INS-KIT3048-001-REVA, June 2025
*Updated recovery data, selectivity data and document ID number. Included use of the AlerTox Polyphenol Additive for some sample preparations.*

INS-KIT3048-001-REVB, February 2026
*Clarify the cross-reactivity statement.*

# Appendix A. Specialized Sample Extraction Procedures

### A.1 For Foods and Drinks Containing Polyphenols, Tannins or Antioxidants

Follow this sample preparation protocol when testing foods and drinks that are rich in polyphenols, including tannins, and antioxidants. Examples are listed in the following table:

| Representative Matrices | | |
|---|---|---|
| Berries | Chocolate | Corn, purple |
| Corn fiber | Coffee | Legumes (e.g., chickpeas, lentils) |
| Soy | Tea | Wine |

**Important**: This procedure is **not** for use with the following kits:

- AlerTox ELISA Crustacean Kit
- AlerTox ELISA Histamine Kit
- AlerTox ELISA Lysozyme Kit
- Wine extracts for the following kits:
  - AlerTox ELISA Casein Kit
  - AlerTox ELISA Ovalbumin Kit

a. For solid samples, maximize the sample homogeneity by finely pulverizing a minimum of 5 g of sample in a mortar, impact mill or similar device.

**Note**: For liquid samples, proceed to Step b.

b. Mix the sample with the AlerTox Polyphenol Additive (Product No. ASY3213) and 1X Extraction & Sample Dilution Buffer, based on the kit used:

i. For AlerTox ELISA Kits except Hazelnut and Pistachio: mix the sample and AlerTox Polyphenol Additive first, then add 1X Extraction & Sample Dilution Buffer (see table below) and mix well.

ii. For the AlerTox ELISA Hazelnut and Pistachio Kits: Dissolve 1 g of AlerTox Polyphenol Additive in 100 mL of 1X Extraction & Sample Dilution Buffer before mixing with the specified amount of sample (see table below).

| Kit | Sample | AlerTox Polyphenol Additive | 1X Extraction & Sample Dilution Buffer |
|---|---|---|---|
| AlerTox ELISA Kits* | 1 g (Step a, solid) | 2 g | 20 mL |
| | 1 mL | 2 g | 19 mL |
| AlerTox ELISA Milk Kit | 0.5 g (Step a, solid) | 1 g | 10 mL |
| | 0.5 mL | 1 g | 9.5 mL |
| AlerTox ELISA Hazelnut and Pistachio Kits | 0.5 g (Step a, solid) | 10 mL | |
| | 0.5 mL | 9.5 mL | |

* i.e., all AlerTox ELISA Kits except those specific for hazelnut, pistachio, milk or those excluded in the Important note above.

c. Incubate for 15 minutes in a preheated water bath at 60 °C (140 °F), shaking the samples every 2 minutes to ensure homogeneity.

d. Centrifuge for 10 minutes at ≥ 2,500 x *g*.

e. If the supernatant is still not completely separated from the particulates, filter the supernatant.

f. Proceed with the *ELISA Procedure* (Section 3.3).

**Important**: The results calculations will not require additional dilution-factor adjustments for this procedure.

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