AlerTox® ELISA Lupine Kit

For the quantitative detection of lupine proteins in food products

REF KIT3057 (96 reactions)

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1. Introduction

Lupines are legumes that are commonly consumed in the Mediterranean and Asia as beans, lupine flour or lupine bran. Their use is increasing in Western countries, especially in gluten-free foods or as a substitute for milk or soy.

Raw lupines contain approximately 36% protein. There are at least 4 allergenic lupine proteins, some of which have structural similarities with allergenic peanut proteins.

In addition to the AlerTox® ELISA Lupine Kit, some manufacturers may want to use AlerTox ELISA Kits for other legumes, such as peanut and soy. The same sample preparation can be used with the AlerTox ELISA tests for lupines, peanuts and soy.

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 Lupine Kit detects and quantifies lupine proteins in foods, such as bread, cakes, dairy products, instant soups, juices, pastries, puddings and other foodstuffs that may be raw, heated or baked. The limit of detection (LOD) is 0.2 ppm (mg of whole lupine seed per kg or L of sample), the limit of quantification (LOQ) is 2 ppm of whole lupine seed (mg/kg or mg/L) and the detection is quantitative between 2 and 30 ppm (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 (%)
Soy flour	0.0065
Clove	0.0009
Fenugreek	0.0006
Cinnamon	0.0005
Adzuki bean	0.0003
Chickpea	0.0003
Cocoa	0.0003
Kidney bean	0.0003
Lentil	0.0003
Nutmeg	0.0003
Pecan	0.0003
Plum	0.0003
Soy lecithin	0.0003

Note: Bean (white), cayenne, cherry, ginger (ground), guar gum, hazelnut, split pea and thyme showed results between 0.5 LOD and 1 LOD 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 Lupine Kit is one in a series of twenty related allergen test kits from Hygiena®. Sixteen different allergens, including lupine, 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 Lupine 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 lupine proteins are bound on the surface of a microtiter plate. Lupine-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 lupine 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-lupine primary antibodies. In a re-sealable foil bag containing a frame and drying agent. Ready to use.	12 strips
2	5 AlerTox Lupine Standards, concentrations: 0 – 2 – 5 – 15 – 30 ppm. Ready to use.	5 x 3 mL
3	Conjugate: Peroxidase-conjugated, anti-lupine 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:

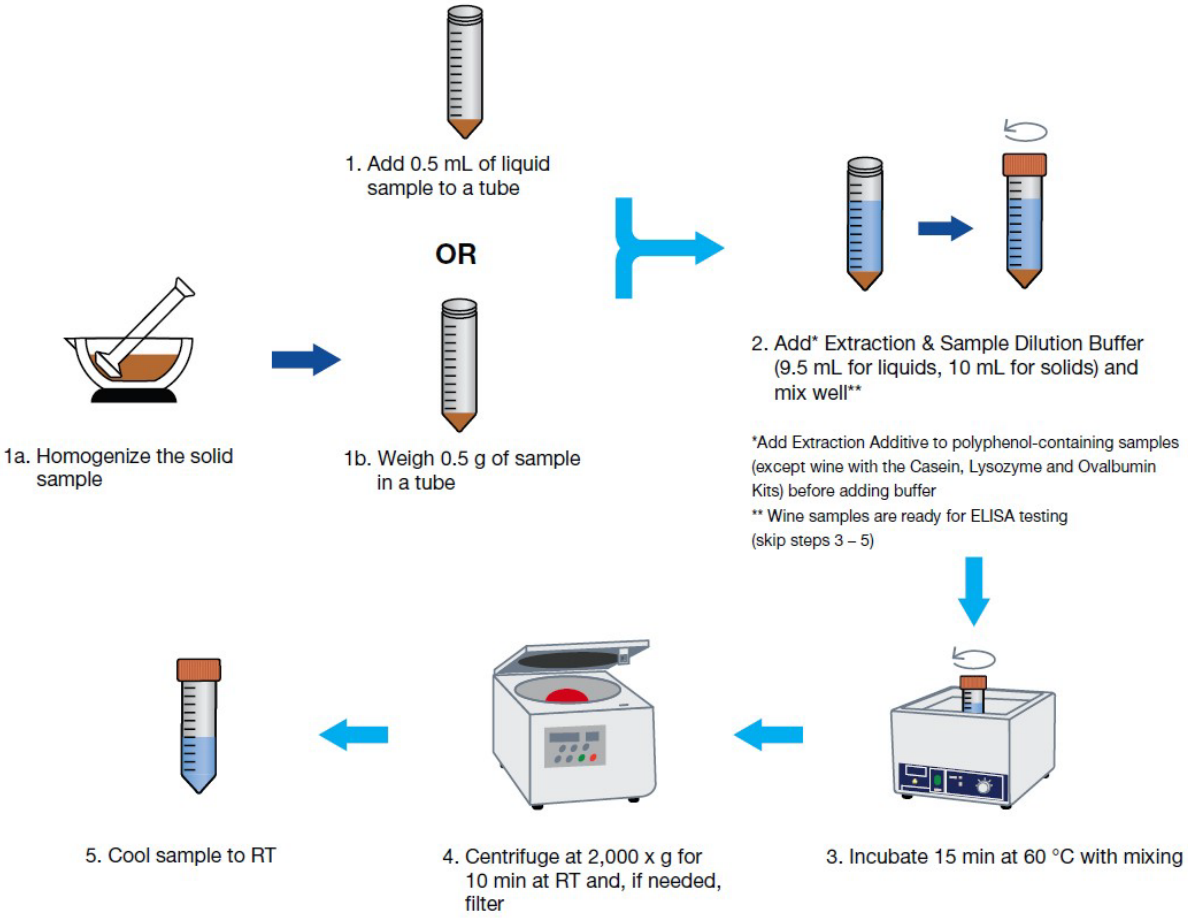
- When opening the foil bag for the first time, be careful not to cut the ziplock off the bag.
- Unused wells must be stored in the foil bag with the drying agent at 2 to 8 °C (36 to 46 °F). Ensure the ziplock on the foil bag is sealed tightly.

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).

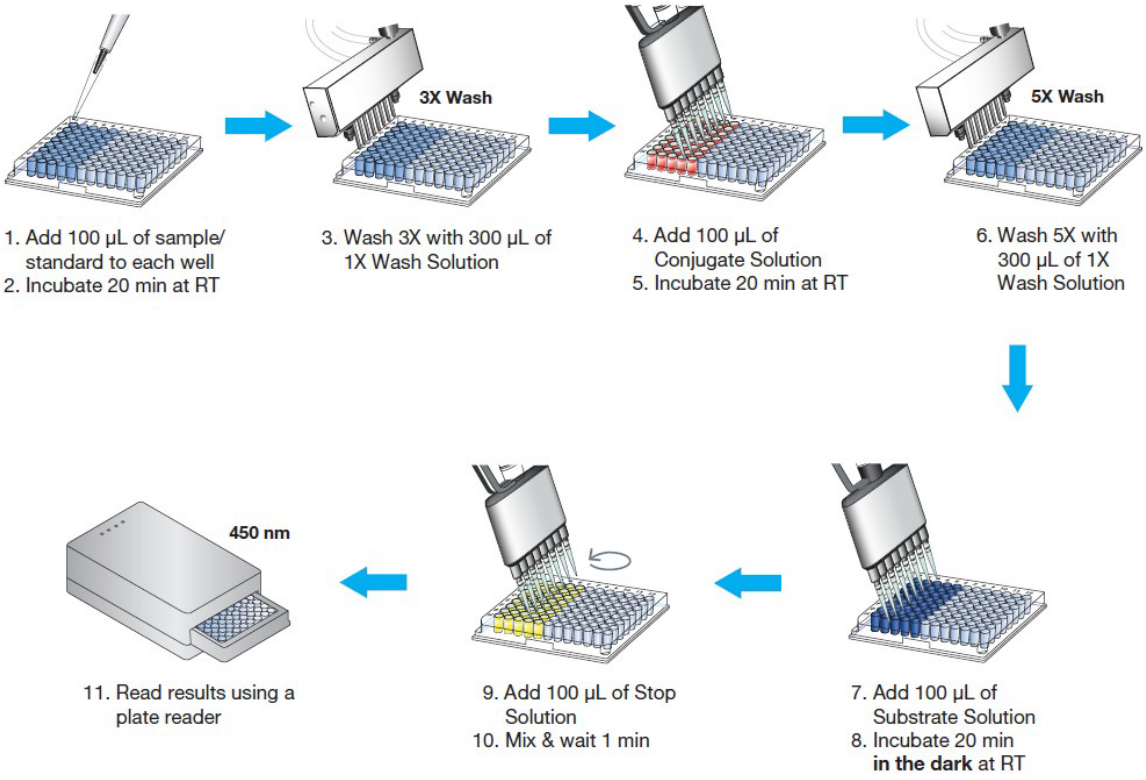
   Important: Do not shake the plate during this incubation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution (0.5 M H_2SO_4) into each well.
10. Gently shake the plate by hand and wait for 1 minute.

    Note: Wells containing blue color turn yellow in the presence of lupine protein.
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 lupine 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 concentrations of whole lupine seed and not lupine protein. See Step 5 below for a conversion factor to calculate lupine protein concentrations.

The standards are prepared for a direct determination of whole lupine seed 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*.

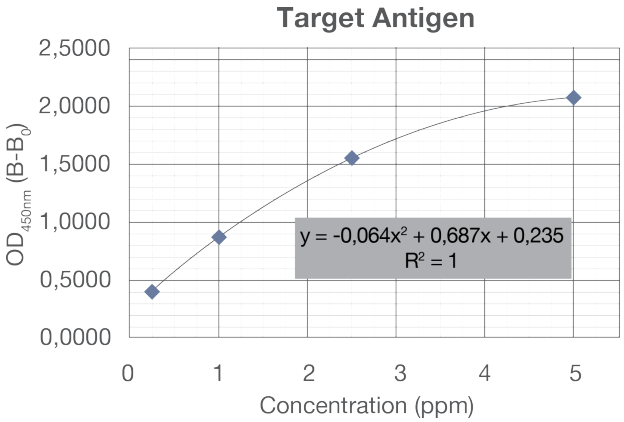
   Important: If the Zero Standard on the plate reader software is defined as the Blank for the calculation of $B - B_0$, skip this step.
3. To create the standard curve, plot the adjusted OD values of standards 1 to 4 on the y-axis versus the concentration of whole lupine seed in ppm on the x-axis. See 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 lupine seed.

   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 lupine seed to ppm of lupine protein, divide the results by 2.8 [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 & 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.)

‡ STI = Soy Trypsin Inhibitor

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 Lupine Kit

6.2.1 Summary of Specifications

Specification	AlerTox ELISA Lupine*
Results	Concentration of whole lupine seed
Limit of Detection (LOD)	0.2 ppm
Limit of Quantification (LOQ)	2 ppm
Standard Range	0 – 30 ppm
Quantification Range	2 – 30 ppm
Calculation Factor†	Lupine protein [1] Divide by 2.8

* ppm = mg of whole lupine per kg or L of sample

† Use the calculation factor to convert the results to the concentration of lupine 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 (%)
Biscuit	113
Croquette	111
Ketchup	98
Orange juice	104
Sausage	99

* 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 Lupine Kit:

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

* Bean (white), cayenne, cherry, ginger (ground), guar gum, hazelnut, split pea and thyme showed results between 0.5 LOD and 1 LOD 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 (2019) Lupins, mature seeds, raw. https://fdc.nal.usda.gov/fdc-app.html#/food-details/172423/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-KIT3057-001-REVA, June 2025
Updated recovery data, selectivity data and document ID number. Included use of the AlerTox Polyphenol Additive for some sample preparations.

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.

Hygiena

Camarillo, CA 93012

USA

www.hygiena.com/support

Manufactured by

Hygiena Diagnóstica España S.L.

P. I. Parque Plata

Calle Cañada Real 31 – 35

41900, Camas (Sevilla), Spain

www.hygiena.com