AlerTox® ELISA Lysozyme Kit

For the quantitative detection of lysozyme in wine, cheese and other food products

REF KIT3044 (96 reactions)

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1. Introduction

Egg is one of the "Big 9" food allergens that require food labeling in many regions of the world to protect sensitized people from potentially life-threatening reactions.

Although some egg yolk proteins may be allergenic, the most common egg allergens are egg white proteins. Egg whites contain 9 – 11% protein, with four allergenic proteins comprising approximately 80% of those proteins. The dominant allergen, ovomucoid (11% of egg white protein), is heat stable. Ovalbumin (54% of egg white protein) and lysozyme (~3% of egg white protein) are not heat stable. The weakest egg white allergen, ovotransferrin/conalbumin (12% of egg white protein), is also not heat stable.

There are three AlerTox® ELISA Kits specific for either ovomucoid, ovalbumin or lysozyme. AlerTox ELISA Lysozyme is designed primarily for testing wine and cheese but can be used with other sample types. AlerTox ELISA Ovalbumin is designed primarily for testing wine but can be used with other sample types. AlerTox ELISA Egg targets ovomucoid and is the all-purpose egg testing kit.

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

1.1 O.I.V. Conformity

The O.I.V. (International Organisation of Vine and Wine) has established the requirements for ELISA test systems to have a limit of detection (LOD) of 0.25 ppm and limit of quantification (LOQ) of 0.50 ppm with a recovery rate between 80% and 105%. The AlerTox ELISA Lysozyme test fulfills these O.I.V. requirements for the detection of lysozyme residue in wine. See *Section 1.2, Test Sensitivity and Specificity*, and *Section 6.2.2, Recovery.*

Highly purified lysozyme is typically used for medical purposes (e.g., vaccine production). Lower-purity lysozyme may be used in food production (e.g., as a wine fining agent) and will have lower recovery rates than more highly purified lysozyme.

1.2 Test Sensitivity and Specificity

The AlerTox ELISA Lysozyme Kit detects and quantifies lysozyme in wine, cheese and other foodstuffs. The limit of detection (LOD) is 2.3 ppb (µg of lysozyme per kg or L of sample), the limit of quantification (LOQ) is 25 ppb lysozyme (µg/kg or µg/L) and the detection is quantitative between 25 and 250 ppb lysozyme (see *Section 6.2.1, Summary of Specifications*, for more details).

Cross-reactivity with other allergenic egg white proteins is noted below:

Cross-Reactive Matrix	Percent Cross-Reactivity (%)
Egg white protein, total	2.2
Conalbumin	< 0.0001
Ovalbumin	< 0.0001
Ovomucoid	< 0.0001

Note: Goat's milk 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.

1.3 Sample Preparation

Important: Please follow these instructions carefully when testing non-wine samples, as there are sample preparation differences compared to most of the AlerTox ELISA Kits. Non-wine sample extracts can only be tested using the AlerTox ELISA Lysozyme assay. Wine sample extracts can be tested with most of the other AlerTox ELISA tests.

See *Section 6.1, Sample Extraction Compatibility*, for more details about other AlerTox ELISA Kits.

1.4 Test Principle

The AlerTox ELISA Lysozyme 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 lysozyme are bound on the surface of a microtiter plate. Lysozyme-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 lysozyme 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-lysozyme primary antibodies. In a re-sealable foil bag containing a frame and drying agent. Ready to use.	12 strips
2	5 AlerTox Lysozyme Standards, concentrations: 0 – 25 – 50 – 125 – 250 ppb. Ready to use.	5 x 3 mL
3	Conjugate: Peroxidase-conjugated, anti-lysozyme 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

- 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
- Distilled water
- ELISA Plate Reader with filter (450 nm) (Absorbance 96 ELISA Reader, Product No. MCH3005, or similar)
- Centrifuge
- NaCl
- Stomacher, Mill, Mortar, Blender, etc.
- Vortex mixer

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

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.

3.1.1.1 Wine Samples

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

   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
Wine	0.5 mL	9.5 mL

3.1.1.2 Cheese and Other Food Samples

1. Dilute the 10X Extraction & Sample Dilution Buffer 1:10 with distilled water to create the 1X solution.
2. Transfer a portion of the 1X solution to a new container and add NaCl to a final concentration of 1 g of NaCl per 10 mL of 1X Extraction & Sample Dilution Buffer.

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	1 g	10 mL (with NaCl) 1 mL (with no NaCl)
Liquid (non-wine)	1 mL	10 mL (with NaCl) 1 mL (with no NaCl)

3.1.2 Washing Solution

1. 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.

   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:

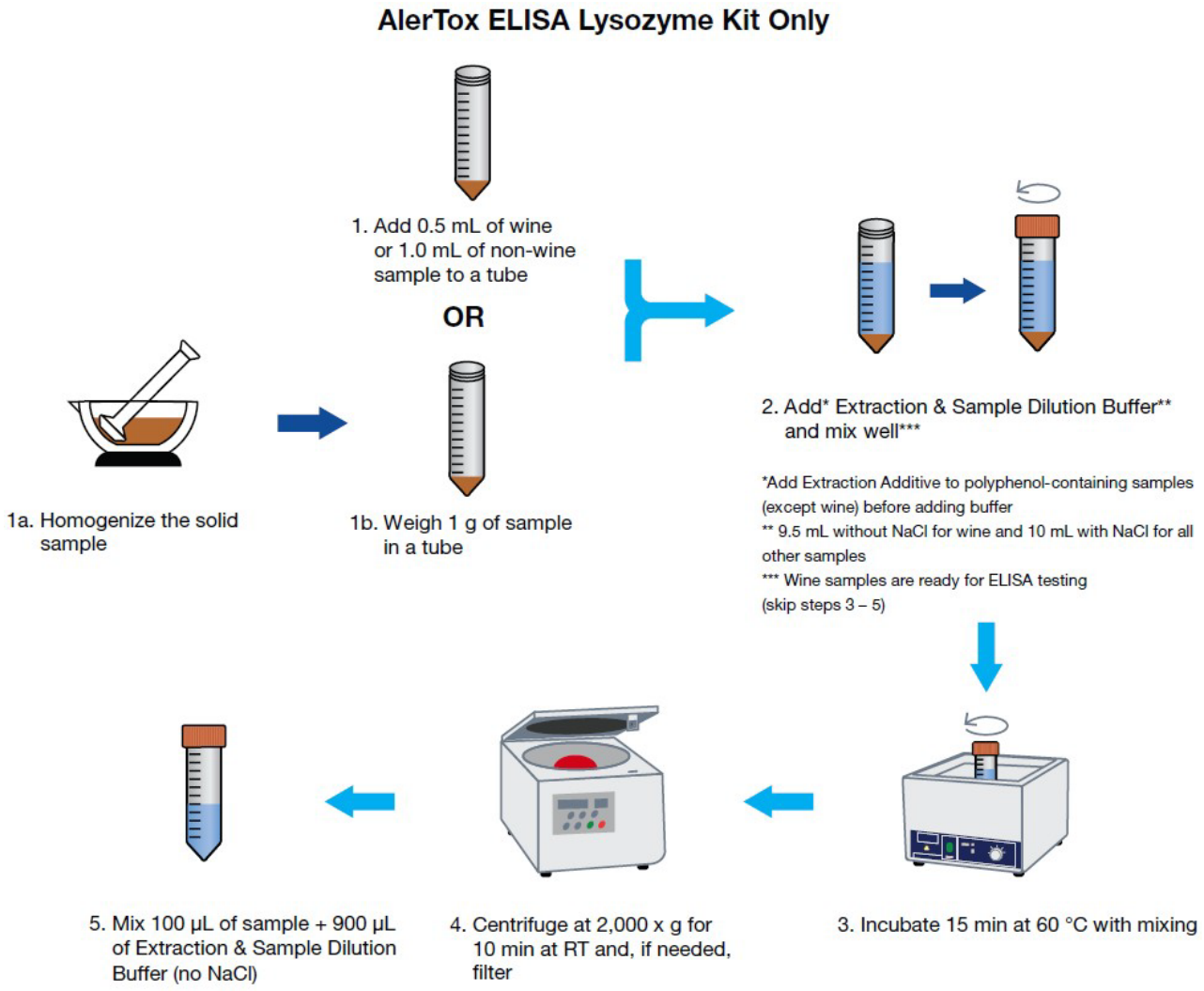
- When opening the foil bag for the first time, be careful not to cut the ziplock off the bag.
- Unused wells must be stored in the foil bag with the drying agent at 2 to 8 °C (36 to 46 °F). Ensure the ziplock on the foil bag is sealed tightly.

3.2 Sample Preparation

1. Resuspend sample in 1X Extraction & Sample Dilution Buffer based on sample type:
   a. For wine samples:
      i. Add 0.5 mL of the wine sample to 9.5 mL of 1X Extraction & Sample Dilution Buffer (no NaCl).
         Note: It is not necessary to adjust the pH of the wine sample because of its dilution in this step.
      ii. Vortex briefly to mix.
      iii. Proceed to ELISA testing (Section 3.3).
   b. For cheese and other solid food 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 1 g of the homogenized mixture in 10 mL of 1X Extraction & Sample Dilution Buffer with NaCl.
   c. For non-wine liquid samples:
      i. Add 1 mL of the liquid sample to 10 mL of 1X Extraction & Sample Dilution Buffer with NaCl.

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. Dilute 100 µL of sample extract (supernatant or filtrate) in 900 µL of 1X Extraction & Sample Dilution Buffer (no NaCl).

3.2.1 Workflow Overview



Important: Sample preparation for this kit is different from other AlerTox ELISA 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.

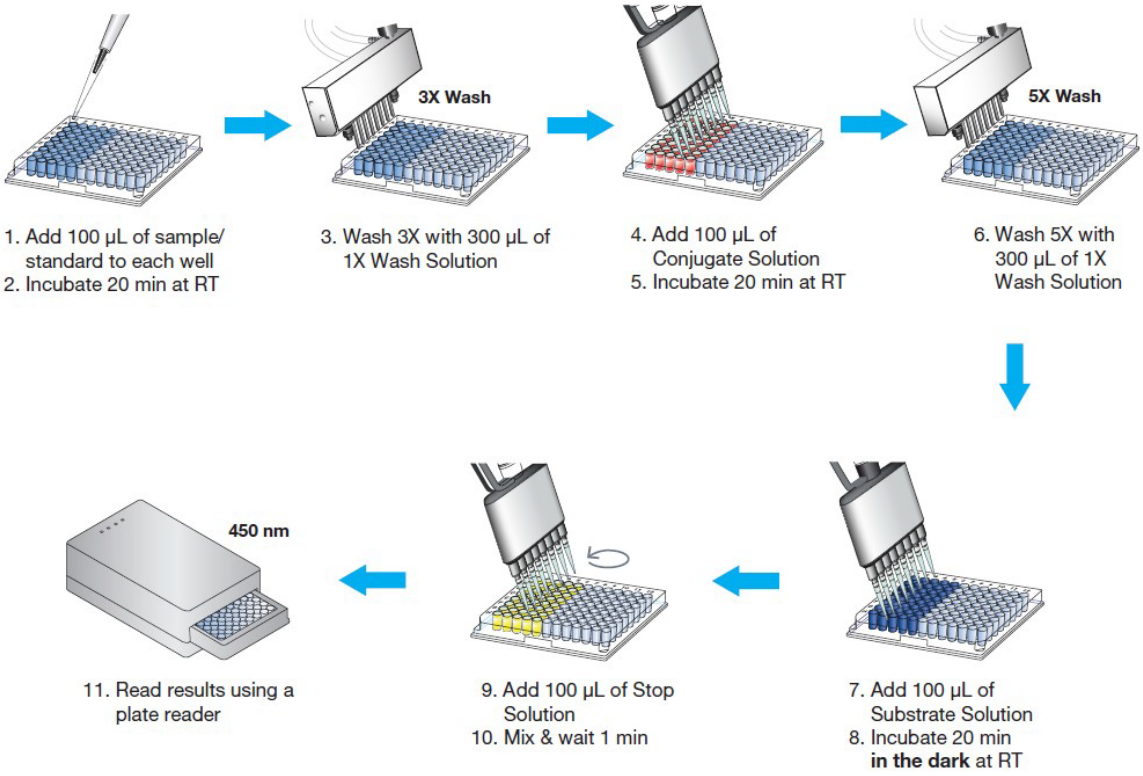
    Note: Wells containing blue color turn yellow in the presence of lysozyme.

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 lysozyme 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 lysozyme concentrations.

The standards are prepared for a direct determination of lysozyme 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 lysozyme concentration in ppb 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 lysozyme concentration.

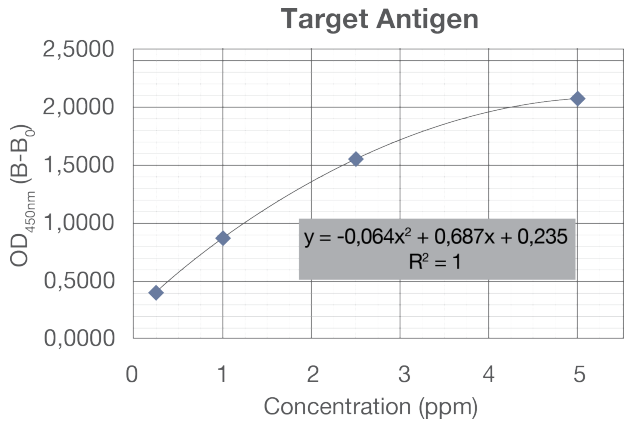
   Notes:

   - For wine samples, it is *not* necessary to multiply the resulting concentration by the dilution factor of 20.
   - For extracts that require NaCl (i.e., cheese and other non-wine food samples), multiply the results by 5 to account for the dilutions.

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.
  - Substrate Solution contains TMB, which is highly toxic if inhaled, ingested or contacts skin. Please refer to the SDS.
  - 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

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.

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

6.2 AlerTox ELISA Lysozyme Kit

6.2.1 Summary of Specifications

Specification	AlerTox ELISA Lysozyme*
Results	Concentration of lysozyme (an egg white protein)
Limit of Detection (LOD)	2.3 ppb
Limit of Quantification (LOQ)	25 ppb
Standard Range	0 – 250 ppb
Quantification Range	25 – 250 ppb

* ppb = µg of lysozyme per kg or L of sample

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 (%)
Cheese	93
Red wine	99
Rosé wine	91

* 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 Lysozyme Kit:

Non-Cross-Reactive Matrices				
Adzuki bean	Almond	Apricot	Barley	Bean, white
Beef (raw)	Beef (cooked)	Brazil nut	Buckwheat	Cabbage, white
Caraway seeds	Cardamom	Carob gum	Carrot	Cashew
Cayenne	Celery	Cherry	Chestnut	Chia seeds
Chicken	Chickpea	Chili	Cinnamon	Clove
Cocoa	Coconut	Cod	Corn	Cumin
Dill	Duck	Fennel	Fenugreek	Flaxseed
Garden cress	Garlic (fresh)	Garlic (granulated)	Gelatin, cow	Ginger (ground)
Ginger (fresh)	Gliadin	Guar gum	Gum arabic	Hazelnut
Horseradish	Kidney bean	Kiwi	Lamb	Leek
Lentil	Lupine	Macadamia	Milk, cow	Milk, goat*
Milk, sheep	Mustard, yellow	Nutmeg	Oats	Onion
Paprika	Pea	Peach	Peanut	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	

***** Goat's milk 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. 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.

9. Contact Information

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

10. Change Index

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

INS-KIT3044-001-REVA, June 2025
Updated recovery data, selectivity data and document ID number.

INS-KIT3044-001-REVB, February 2026
Clarify the cross-reactivity statement.

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