AlerTox® ELISA Soy Kit

For the quantitative detection of soy proteins (soy trypsin inhibitor) in food products

REF KIT3047 (96 reactions)

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1. Introduction

Soybeans are considered one of the “Big 9” food allergens and are members of the legume family along with peanuts, lupines, peas, lentils and beans. The prevalence of soy allergies is approximately 0.3–3%, affecting primarily children but sometimes persisting into adulthood. Although peanut allergy sufferers are not more likely to have soy allergies, people with soy allergies are more likely to have peanut allergies and may also be cross-reactive to birch pollen allergens.

Soybeans have become an important food ingredient because of their high nutritional value, but they are also used in cosmetics and other industrial applications. Soy contains 30–50% protein and at least 16 allergenic proteins, including seed storage proteins (e.g., conglycinin and glycinin) and defense proteins (e.g., soy trypsin inhibitor, STI). STI constitutes approximately 6% of soy protein. STI detection can be used to monitor soy content in foods and determine the efficiency of STI heat inactivation used by some food manufacturers to neutralize STI in soy flour and other products.

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 Soy Kit detects and quantifies soy proteins based on STI in foods, such as cakes, chocolate, desserts, pralines, puddings and other foodstuffs, which may be raw, heated, cooked or baked. The limit of detection (LOD) is 16 ppb (µg of STI per kg of sample), and the limit of quantification (LOQ) is 50 ppb STI (µg/kg). The detection is quantitative between 50 and 600 ppb STI (µg/kg; see *Section 6.2.1, Summary of Specifications*, for more details).

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

Lot Number	Cross-Reactive Matrix	Percent Cross-Reactivity (%)
Before 340225	Rapeseed	0.00001
340225 and later	Adzuki bean	0.00001
	Fenugreek	0.000005

Note: For kits with lot number 340225 and later, kidney bean showed results between 0.5 LOQ and 1 LOQ and may provide values above the LOQ.

For additional data, see *Section 6.2.2, Recovery*, and *Section 6.2.3, Non-Cross Reactivity*.

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 Soy Kit is one in a series of twenty related allergen test kits from Hygiena®. Sixteen different allergens, including soy, 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 Soy 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 STI are bound on the surface of a microtiter plate. STI-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 STI 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, which 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-STI primary antibodies. In a re-sealable foil bag containing a frame and drying agent. Ready to use.	12 strips
2	5 AlerTox STI Standards, concentrations: 0 – 50 – 150 – 300 – 600 ppb. Ready to use.	5 x 3 mL
3	Conjugate: Peroxidase-conjugated, anti-STI 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:

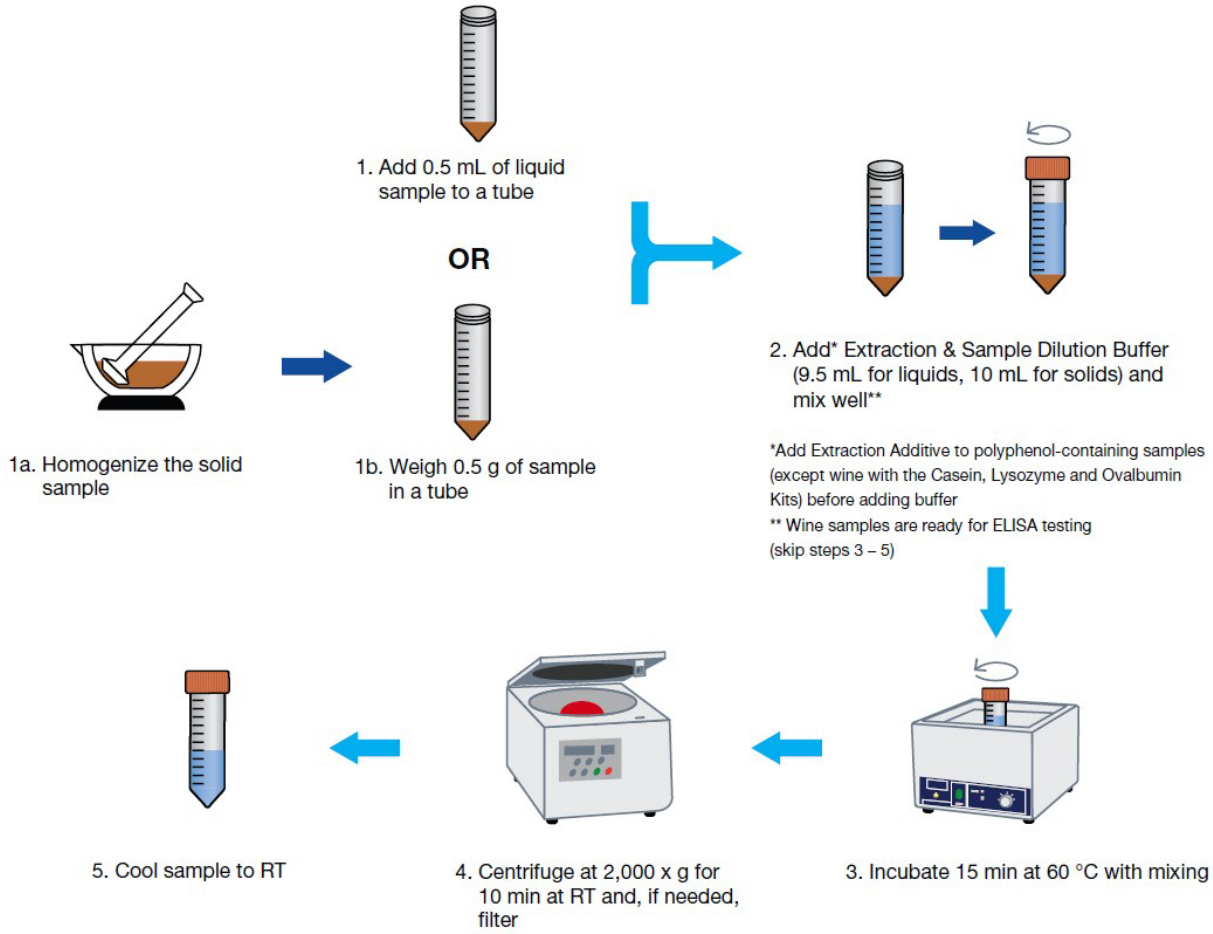
- When opening the foil bag for the first time, be careful not to cut the ziplock off the bag.
- Unused wells must be stored in the foil bag with the drying agent at 2 to 8 °C (36 to 46 °F). Ensure the ziplock on the foil bag is sealed tightly.

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.

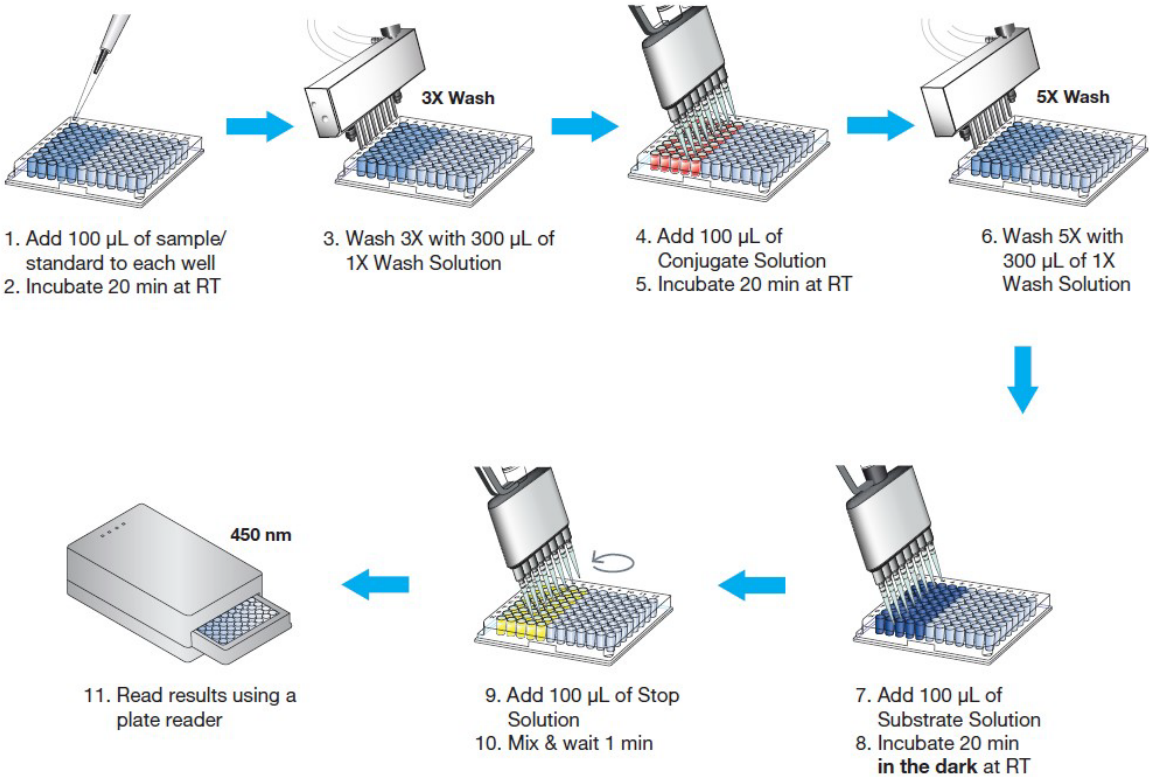
    Note: Wells containing blue color turn yellow in the presence of STI protein.

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 STI 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 STI protein concentration. See Step 5 below for conversion factors to calculate concentrations of other soy products.

The standards are prepared for a direct determination of STI 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 STI 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 concentration of STI.

   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.

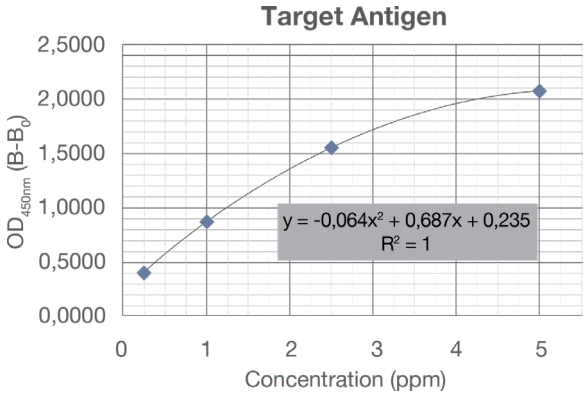
5. To convert ppb of STI to ppb of a soy product, multiply by the appropriate conversion factor listed in the following table:

Matrix	Conversion Factor (Multiply)	
	Lot numbers before 340225	Lot number 340225 and later
Soy flour (unroasted)	42	52
Soy flour (roasted)	470	250
Soy protein isolate (90%)	864	440
Soy milk	2,500	3,700
Textured soy (granulate)	3,080	4,550
Tofu	50,000	7,000

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 Soy Kit

6.2.1 Summary of Specifications

Specification	AlerTox ELISA Soy*		
Results	Concentration of soy trypsin inhibitor		
Limit of Detection (LOD)	16 ppb		
Limit of Quantification (LOQ)	50 ppb		
Standard Range	0–600 ppb		
Quantification Range	50–600 ppb		
Calculation Factors†	*Food types*	*Lot numbers before 340225, Multiply by*	*Lot number 340225 and later, Multiply by*
	Soy flour (unroasted)	42	52
	Soy flour (roasted)	470	250
	Soy protein isolate (90%)	864	440
	Soy milk	2,500	3,700
	Textured soy (granulate)	3,080	4,550
	Tofu	50,000	7,000

* ppb = µg of STI per kg of sample

† Use the calculation factor to convert the results to the concentration of different food types.

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	77
Cookies	106
Cornflakes	100
Ice cream	77
Instant Soup	90
Sausage	96

* 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 Soy Kit:

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

* Adzuki bean: For kits with lot numbers earlier than 340225, adzuki bean is non-cross-reactive. For kits with lot number 340225 and later, adzuki bean has a cross-reactivity of 0.00001%.

† Fenugreek: For kits with lot numbers earlier than 340225, fenugreek is non-cross-reactive. For kits with lot number 340225 and later, fenugreek has a cross-reactivity of 0.000005%.

‡ Kidney bean: For kits with lot numbers earlier than 340225, kidney bean is non-cross-reactive. For kits with lot number 340225 and later, kidney bean showed results between 0.5 LOQ and 1 LOQ and may provide values above the LOQ.

§ Rapeseed: For kits with lot numbers earlier than 340225, rapeseed has a cross-reactivity of 0.00001%. For kits with lot number 340225 and later, rapeseed is non-cross-reactive.

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-KIT3047-001-REVA, June 2025
Updated recovery data, selectivity data and document ID number. Included use of the AlerTox Polyphenol Additive for some sample preparations.

INS-KIT3047-001-REVB, November 2025
Added cross-reactivity and calculation factors for kits with lot numbers starting with 340225.

INS-KIT3047-001-REVC, 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 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 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 (Step b.ii)	
	0.5 mL	9.5 mL (Step b.ii)	

* 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

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