ENHANCED ASSAY

Soy Protein Residue

Product Code: ESSOYPRD - 48

Microwell ELISA For Laboratory Use Only **Store Between 2 - 8°C**

For screening for the presence of Soy Protein Residues in Food Products and Environmental Samples.

Directions For Use

Intended Use

The ELISA SYSTEMS Soy Protein Residue assay is an enzyme-linked immunosorbent assay (ELISA) that may be used to screen appropriate food products for the presence of soy protein material caused by cross-contamination with soy products and residues. Samples that have been subjected to prolonged high temperature and pressure treatments (such as in canning operations), hydrolysis or fermentation, may not be suitable for analysis using this test kit. Please discuss with your ELISA SYSTEMS representative regarding the suitability of this kit for these samples.

Background

Although the incidence of allergy to Soybean proteins is quite low in comparison with other major food proteins, the gradually increasing consumption of Soybean products makes the identification and characterization of major Soy allergens a focus of investigation (Helm et al 2000).

The major allergens of soybean have not been as well characterized as peanut allergens, however, two soy proteins have been identified as antigenic (Eigenmann et al.1996). Soy Trypsin Inhibitor and other Soy proteins were chosen for the detection of Soy protein residue material for this assay.

This assay is based on comparison to Soy Flour Protein Concentrations

Please note: A special extraction solution is required for samples containing Polyphenols, including Dark Chocolate, Wine, Fruit Juices, Herbs, and Tannins. (Product Code: ESADDSOL).

Principle of Procedure

The ELISA SYSTEMS Soy Protein Residue ELISA is a double antibody (sandwich) ELISA utilizing specific Anti-Soy Trypsin Inhibitor and other Soy protein antibodies coated onto microwells. After addition of the sample, the Enzyme Conjugate, then the TMB Substrate, a positive reaction (indicating the presence of Soy protein) produces a blue colour. Addition of the Stop Solution ends the assay and turns the blue colour to yellow. The results may be read visually or with an ELISA reader.

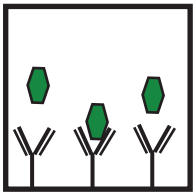
Note: The level of soy proteins present in a product will vary according to the ingredients and the manufacturing process. This test may not detect soy protein material that has been significantly treated or altered through processes such as high temperature and/or pressure, fermentation or hydrolysis. If no soy protein is detected, this cannot conclusively indicate there is no absolute trace of soy material present. The supplied positive controls serve to indicate the approximate levels of soy flour protein present in the sample. This factor must be taken into consideration when assessing the potential total soy protein concentration and the allergenic issues associated with the sample being tested. The results of the testing are only applicable to the portion of the sample product tested and to this extent, ELISA SYSTEMS cannot guarantee that soy material is, or is not, present in the untested portions of the sample product.

The assay is designed for screening purposes.
Any sample returning a positive result should be regarded as a presumptive result and confirmation or further testing should be performed.

How the ELISA SYSTEMS Soy Protein Residue test works:

Step 1

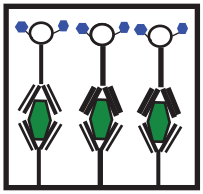
Sample is added



The test sample is added and if Soy residue is present, it will bind to the specific antibodies.

Step 2

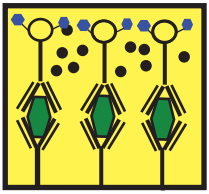
Antigen-Antibody Complex



Enzyme-labelled Conjugate is added and binds to the captured Soy residue to form a “Sandwich”.

Step 3

Coloured End-Point



TMB Substrate is added, which is converted in the presence of the Enzyme Conjugate to form a blue colour if Soy residue is present in the sample.

A yellow colour is formed once Acid is added to stop the reaction.

Reagents Supplied

Test Strips: microwells containing anti-Soy Protein antibodies – 48 wells.
Test strip holder: One (1)

Negative Control Standard: One (1) vial containing 1.7 ml of a buffered base.

Positive Control Standards:
One (1) vial containing 1.7 ml of Soy Protein in a buffer to provide a Control value of 2.5 ppm
One (1) vial containing 1.7 ml of Soy Protein in a buffer to provide a Control value of 5.0 ppm
One (1) vial containing 1.7 ml of Soy Protein in a buffer to provide a Control value of 10.0 ppm
One (1) vial containing 1.7 ml of Soy Protein in a buffer to provide a Control value of 25.0 ppm

Enzyme Conjugate:
One (1) bottle containing 7 ml of Peroxidase conjugated anti-Soy Protein polyclonal antibodies with preservative.

Substrate: One (1) bottle containing 7 ml of a stabilized Tetramethylbenzidine (TMB).

Wash Buffer Solution concentrate (20x): Three (3) bottles containing 25 ml each of concentrated wash buffer solution with Preservative.

Extraction Solution concentrate (20x): Three (3) bottles containing 25 ml each of concentrated extraction solution with Preservative.

Stop Solution: One (1) bottle containing 7 ml of 1M Phosphoric acid.
(CAUTION THIS SOLUTION IS ACIDIC) Avoid contact of this solution with eyes and skin. In case of skin contact, wash immediately with copious amounts of water. A mild soap should be used. In case of eye contact, flush generously for at least 15 minutes with water. Seek urgent medical attention if the irritation persists or is severe.

Additional Materials Required

Suitable clean containers for use in the sample extraction procedure. Do not use Polystyrene containers as these could absorb protein from the extract. We suggest low binding capacity plastic disposable containers.

Pipette: 100 microlitre, Disposable tips. A 200–1000 microlitre pipette, if available, for aliquoting reagents. Clean test tubes or small microtubes for aliquotting the Enzyme
Conjugate and Substrate volumes prior to use. Timer. Plastic Wash bottle with a fine tip. Data record sheets. Marking-pen, fine tipped. Water Bath or a similar system, capable of heating and holding the extraction buffers and samples at 60^{0}C. Paper towels. Distilled or Deionized water. Laboratory Vortex machine. Blender, Grinder, Stomacher, Ultraturrax or similar devices for sample preparation.
Disinfecting Solution or a system for Biological waste removal.
Optional for Screening, but required for Quantitative analysis: Microplate reader, preferably capable of reading bichromatically at 450/620-650 nm.

Please note: A special extraction solution is required for samples containing Polyphenols, including Dark Chocolate, Wine, Fruit Juices, Herbs, and Tannins. (Product code: ESADDSOL).

Precautions

Wash Solution and Extraction Solution concentrates may precipitate during refrigerated storage but will dissolve upon warming. Any precipitates must be fully dissolved prior to diluting out to the final working strength.

You should not use more than two strips of 8 wells at a time unless you use a multichannel pipette to add the samples, controls and other reagents, otherwise timing errors may occur.

Do not add azides to the samples or any of the reagents. Controls and some reagents contain a preservative.

Treat all reagents and samples as potentially allergenic materials.

The pH of the samples in extraction buffer ***prior*** to the extraction procedure should be in the range pH 6.8 - 7.4.

Do not pipette directly from the Substrate and Enzyme Conjugate bottles as this will contaminate these solutions. Always determine the required volumes of these reagents and dispense the volumes required accordingly into clean test tubes just prior to use. Do not pour or return unused Enzyme Conjugates or Substrate back into their bottles. All pipette volumes should be ± 1 microlitre.

Always firmly reseal the foil bag containing the antibody coated strips, to prevent moisture contamination.

Ensure all glassware, plasticware and storage bottles have been thoroughly cleaned to prevent any cross contamination. If cleaning is inadequate traces of allergenic material may remain from other test kits or previous test runs. Do not use Polystyrene containers as these could absorb protein from the extract. We suggest low binding capacity plastic disposable containers.

Storage Conditions

Reagents, strips and bottled components:
Store between 2 - 8ºC. ***DO NOT FREEZE ANY OF THE KIT COMPONENTS.***

Squeeze bottle containing diluted wash buffer may be stored at room temperature.
Avoid exposure of the kit and the components to direct sunlight at any time, as some reagents are light sensitive.

Reagent Preparation

Wash Buffer

Remove the cap and add contents of one bottle of the 20x Concentrate to distilled or deionized water to make a final volume of 500ml. Mix gently. Transfer contents of diluted wash buffer into a squeeze bottle (small tip bottle). For long term storage, label the storage bottle containing the diluted Wash buffer with the kit lot number and kit expiry date.

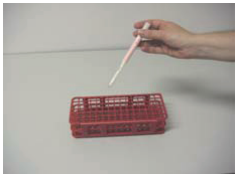
Extraction Solution

Remove the cap and add contents of one bottle of the 20x Concentrate to distilled or deionized water to make a final volume of 500ml. Mix gently. Transfer contents of diluted Extraction solution into a storage bottle. For long term storage, label the storage bottle containing the diluted Extraction solution with the kit lot number and kit expiry date.

Food Allergen/Residue Swab Sampling Protocol

Select a new swab tube.

Label the swab tube carefully, to identify the sample.



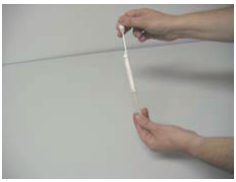
Place the swab tube in a suitable rack or holder.

Open a tube of swab wetting solution.

Pre-wet the swab by inserting the tip of the swab into the tube of wetting solution.

Remove excess moisture from the swab tip by pressing on the inside of the swab tube.

Swab the surface using a cross-hatch technique or according to your own protocol.



Place the swab tip into the labelled swab tube.

Cap or seal the swab tube.

Store the sealed samples as suggested by the laboratory until ready for collection and the extraction procedure.

Sample Preparation

A representative sample(s) must be taken from the product. The sample must be blended to a fine consistency to provide a homogeneous mixture. For each sample, measure the volume required of the diluted Extraction Solution and warm to 60°C.

Please note: A special extraction solution is required for samples consisting of or containing Polyphenols, including Dark Chocolate, Wine, Fruit Juices, Herbs, and Tannins. (Product Code: ESADDSOL).

FOR SOLID SAMPLES

Weigh out 5 grams of finely blended/ground sample into a suitable clean container for extraction purposes. Add 50ml of the pre-warmed diluted Extraction Solution. A suitable blender, Vortex machine, or a similar mixing device should be used to allow complete mixing.

A ratio of 1 part sample plus 10 volumes of the prepared Extraction Solution must be used. Blend or mix until the sample is homogeneous and only minimal clumps are present. Complete mixing to remove clumps so as to ensure consistent results.
The pH of the sample in the extraction buffer should be in the range of 6.8 - 7.4.

Place into a water bath at 60°C for 15 minutes, with shaking/mixing for one minute every 5 minutes. After the completion of the incubation and mixing stage, remove from the water bath and allow to settle and reach room temperature (20-25°C). Filter the extract through Filter paper (medium fast grade) or similar and collect the filtrate. Alternatively, the samples may be centrifuged and the supernatant collected.
The filtrate or the supernatant should be mixed well. This is the sample to be tested on the kit.

FOR LIQUID SAMPLES

A ratio of 1 part sample plus 9 parts of the prepared Extraction Solution must be used for liquid samples. For most samples, place 5 ml of sample into a suitable blender, bottle or similar device and add 45ml of the pre-warmed diluted Extraction Solution. If samples are considered fully homogeneous and representative of a larger batch, a smaller sample volume may be used, as long as the 1 + 9 ratio is maintained.
The pH of the sample in the extraction buffer should be in the range of 6.8 - 7.4.

Place into a water bath at 60°C for 15 minutes, with shaking/mixing for one minute every 5 minutes. After the completion of the incubation and mixing stage, remove from the water bath and allow to settle and reach room temperature (20-25°C). Filter the extract through Filter paper (medium fast grade) or similar and collect the filtrate. Alternatively, the samples may be centrifuged and the supernatant collected.
The filtrate or the supernatant should be mixed well. This is the sample to be tested on the kit.

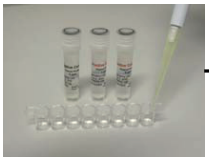
FOR SWAB SAMPLES

Select a new Swab tube and label carefully. Place 1ml of the diluted Extraction Solution into a clean test tube, (not the Swab tube) or contact your ELISA SYSTEMS Distributor for pre-filled swab wetting tubes. Premoisten the Swab tip and remove excess liquid by drawing up along the inside of the tube with slight pressure. Swab the appropriate area according to your protocol. Place the Swab back into the labelled Swab tube and seal. Extract and test as soon as is possible.

To extract the material, add 1 ml of the appropriate, diluted Extraction Buffer to the Swab tube and place the sealed Swab tube into a water bath at 60°C for 15 minutes, with shaking or mixing for one minute every 5 minutes. Vortexing is recommended. After the completion of the incubation and mixing stage, remove from the water bath and allow to settle and cool to room temperature (20-25°C). Filtration is normally not required. Decant the extract into a small test tube and mix well. This is the sample to be tested on the kit.

Swab samples should be regarded as an indication of the presence/absence of the allergen protein(s) detected by this kit. Swab samples cannot be used to quantify the absolute amount of allergen proteins, but can be used as a general indication for monitoring of the levels present.

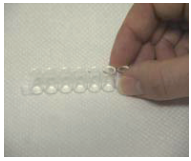
Food Allergen Residue ELISA Protocol



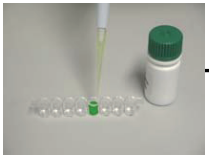
Add 100 microlitres of Controls and Samples to their allocated Antibody-coated wells.
Mix all wells for 10 seconds by gentle shaking on a flat surface.
Incubate for 30 minutes.

Dump liquid from wells.

Wash wells thoroughly five times with wash buffer.



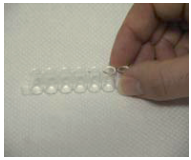
Tap wells firmly onto absorbent paper towel.



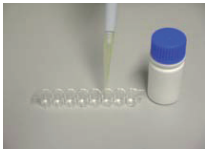
Add 100 microlitres of the Green Conjugate Solution to each well.
Mix all wells for 10 seconds by gentle shaking on a flat surface.
Incubate for 30 minutes.

Dump liquid from wells.

Wash wells thoroughly five times with wash buffer.



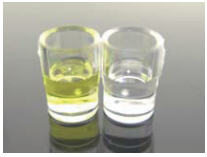
Tap wells firmly onto absorbent paper towel.



Add 100 microlitres of the Substrate Solution to each well.
Mix all wells for 10 seconds by gentle shaking on a flat surface
Incubate for 15 minutes.

DO NOT WASH

Add 100 microlitres of the Stop Solution to each well.
Mix all wells for 10 seconds by gentle shaking on a flat surface.



Read results visually, comparing with the colour of the control well(s).
The results can be read on a microplate/strip reader.
Results must be read within 30 minutes

ELISA Systems

Test Procedure - Qualitative Screening Method.

You should not use more than two strips of 8 wells at a time unless you use a multichannel pipette to add the samples, controls and other reagents, otherwise timing errors may occur.

Photocopy the Sample Coding Sheet supplied on Page 11.
Ensure all kit components are at room temperature (20 – 25^{o}C) prior to commencing this assay.
The negative and at least 1 positive control must be included each time the assay is run.
For screening purposes, use the 2.5 ppm control as the positive control (suggested).
The choice of the positive control may depend on the sample matrix being tested.

Mix the Controls thoroughly prior to each use, preferably with a laboratory vortex machine.

Calculate the amount of the Enzyme Conjugate and the Substrate required. As a guide, find the number of wells to be used in the test, multiply by 0.1ml and add about 10% of this as extra volume for pipetting purposes. Add the required amount of each of these reagents to clean, labelled tubes for use when required in the following steps. (The Substrate should not be pipetted from the bottle until immediately prior to use to prevent prolonged exposure to light).

1. Break off the number of wells needed (number of samples plus the number of controls) and place in the strip holder. Use a fine tipped marker pen to place an identification mark on one end of each strip to allow for correct identification of the wells in the strip holder. Do not mark the bottoms of the wells. Refer to your Sample Coding Sheet for the position of the Samples and the kit controls.

2. Add 100 microlitres of the extracted test sample(s) to the correct test well(s) starting in column 1.

3. After all the samples have been added correctly to the wells in accordance with your sample coding sheet, add 100 microlitres of the Negative control followed by 100 microlitres of the selected Positive control(s) to the appropriate well.
   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 30 minutes, then wash.#

4 Add 100 microlitres of the Enzyme Conjugate (Green Solution) to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 30 minutes, then wash.#

5. Add 100 microlitres of the Substrate Solution to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 15 minutes. **DO NOT WASH AT THIS STAGE**

6. Add 100 microlitres of the Stop Solution to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.

7. Read results visually or on a microplate reader, preferably using a bichromatic reading, with the filters set at 450nm (primary) & 620-650nm (secondary/reference).
   Read Results within 30 minutes of the addition of the Stop Solution.

Each washing consists of dumping the contents of the wells into a sink or an appropriate container. Use the diluted wash buffer to fill each well to overflowing, flicking out the contents thoroughly and refilling the wells, for a total of 5 times. Then tap wells thoroughly by patting against absorbent paper towels. Samples with sticky particulate matter may require more thorough washing than other samples. The potential exists for false positive results if the sample and each reagent are not thoroughly washed from the well before addition of subsequent reagents.

Test Procedure - Quantitative Screening Method.

You should not use more than two strips of 8 wells at a time unless you use a multichannel pipette to add the samples, controls and other reagents, otherwise timing errors may occur.

Photocopy the Sample Coding Sheet supplied on Page 11.
Ensure all kit components are at room temperature (20 – 25^{o}C) prior to commencing this assay.
Controls for a Standard Curve must be included each time the assay is run.
Mix the Controls thoroughly prior to each use, with a laboratory vortex machine.

Calculate the amount of the Enzyme Conjugate and the Substrate required. As a guide, find the number of wells to be used in the test, multiply by 0.1ml and add about 10% of this as extra volume for pipetting purposes. Add the required amount of each of these reagents to clean, labelled tubes for use when required in the following steps. (The Substrate should not be pipetted from the bottle until immediately prior to use to prevent prolonged exposure to light).

1. Break off the number of wells needed for the samples and place in the strip holder.
   Break off the number of wells for the controls and place in a separate Control Column in the well holder. Use a fine tipped marker pen to place an identification mark on one end of each strip to allow for correct identification of the wells in the strip holder. Do not mark the bottoms of the wells.

2. Add 100 microlitres of the extracted test sample(s) to the appropriate test well(s).

3. Add 100 microlitres of the Negative control to well #1 of the Control Column
   Add 100 microlitres of the 2.5 ppm Positive control to well #2 of the Control Column
   Add 100 microlitres of the 5.0 ppm Positive control to well #3 of the Control Column
   Add 100 microlitres of the 10.0 ppm Positive control to well #4 of the Control Column
   Add 100 microlitres of the 25.0 ppm Positive control to well #5 of the Control Column

   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 30 minutes, then wash.#

4. Add 100 microlitres of the Enzyme Conjugate (Green Solution) to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 30 minutes, then wash.#

5. Add 100 microlitres of the Substrate Solution to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 15 minutes. **DO NOT WASH AT THIS STAGE**

6. Add 100 microlitres of the Stop Solution to each well.
   Mix wells by moving strip holder gently sideways for 10 seconds.

7. Read results visually or on a microplate reader, preferably using a bichromatic reading, with the filters set at 450nm (primary) & 620-650nm (secondary/reference).
   Read Results within 30 minutes of the addition of the Stop Solution.

Each washing consists of dumping the contents of the wells into a sink or an appropriate container. Use the diluted wash buffer to fill each well to overflowing, flicking out the contents thoroughly and refilling the wells, for a total of 5 times. Then tap wells thoroughly by patting against absorbent paper towels. Samples with sticky particulate matter may require more thorough washing than other samples. The potential exists for false positive results if the sample and each reagent are not thoroughly washed from the well before addition of subsequent reagents.

Interpretation of Results

This assay is based on comparison of colour developed in sample wells with colour developed in the Control Standards. Results are expressed as ppm or mg of allergen detected per kg sample.

NOTE: The negative control, as well as some samples, may show some slight yellow colour. Please refer to the enclosed kit performance criteria as set out in the accompanying Certificate of Analysis for each specific lot number. The positive control(s) should be a distinct yellow colour. If there is no yellow colour in the positive control, the test should be regarded as invalid and should be repeated. If the positive control again shows no colour, then contact ELISA SYSTEMS immediately.

Interpretation is based on the suggested extraction/dilution protocol. **The values listed for the kit standards already take into consideration the normal extraction dilution used in this method. Therefore, no additional multiplication factors of the kit standards should be used, unless the samples are extracted using a different dilution protocol to that listed in the kit method.**

For Quantitative assays, the sample should fall in the range of the standards supplied (2.5 - 25 ppm Soy Flour Protein) to provide the most accurate result. The sample may need to be diluted to achieve this result and if this occurs, remember to apply the dilution factor used in the calculation of the result.

Results are for screening purposes. **Any sample returning a Positive result should be regarded as a Presumptive result and confirmation or further testing should be performed.** All results should be interpreted as part of a HACCP plan for Food Allergens. Please refer to the information on Page 12.

Qualitative Method

Visual or ELISA Reader

The lowest supplied positive control is recommended as the cut-off for screening purposes. Compare the colour or Optical Density (OD) of the sample well against the colour or OD of the positive control well. Any sample well that has a yellow colour (OD) of the same or greater intensity than the positive control, is suspected to contain Soy protein at a level above the chosen control sample.

Quantitative Method

ELISA Reader

Zero the ELISA Reader. Read all wells using a microstrip reader, preferably with bichromatic filters at 450nm and 620-650nm.

Plot a Standard Curve using the OD values of the Control Standards (OD vs Concentration). Read the concentration of the test samples from this curve.

The lower limit of Quantitation for this assay is the value of the lowest Positive Control which is 2.5 ppm Soy Flour Protein. Results that indicate a value of greater than 1.25 ppm should be further investigated.

Quality Control

The use of a kit positive and kit negative control allows validation of kit stability. For a valid test, the kit controls should correspond to the kit performance criteria as set out in the accompanying Certificate of Analysis for each specific lot number. Should the values fall outside these ranges as listed in the Certificate of Analysis, please contact ELISA SYSTEMS.

Trouble Shooting

Problem: Negative control has substantial colour development.
Correction: Washings were insufficient. Repeat test with more vigorous washings.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Sample Coding Sheet

Assay Name _____ Date Performed _____

Operator _____ Strip washing Method: [ ] Manual or [ ] Machine

Room Temperature_____ Laboratory Incubator used ? Yes/No Temperature of Incubator _____

Comments _____

_____

Assay Incubation Times: Step 1 _____ Step 2 _____ Step 3 _____ Step 4 _____

Caution: Foods can represent a diverse range of components, from simple ingredients to very complex formulations, depending on the nature of the food matrix and the way in which the food has been prepared or processed.

There are many combinations of formulations, additives, processes, treatments etc, that may affect the food sample and even the proteins being tested. This must be considered in the application of this assay for the samples being tested and in the interpretation of the results. Choose the most appropriate Positive control for your screening. This may depend on the sample matrix being tested.

The results of the testing are only applicable to the portion of the sample product tested and to this extent, ELISA SYSTEMS cannot guarantee that Soy material is, or is not, present in the untested portions of the sample product.

Swab samples should be regarded as an indication of the presence/absence of the allergen protein(s) detected by this kit. Swab samples must not be used to quantify the absolute amount of allergen proteins, but should be used as a general indication for monitoring of the levels present.

Not all samples may be suitable for use with this assay. Please discuss your questions with your ELISA SYSTEMS representative.

Assays should be performed in the temperature range of 20 to 25^{o}C.

References.

1. Helm.,R.M., Cockrell,G., Connaughton,C., West,C.M., Herman,E., Sampson,H.A., Bannon, G.A., Burks,W.A., Mutational analysis of the IgE-binding epitopes of P34/Gly m Bd 30K J. Allergy Clin Immunol. 105 378-84.
2. Eigenmann.P.A., Burks,A.W., Bannon,G.A., Sampson,H.A. (1996) Identification of unique peanut and soy allergens in sera absorbed with cross-reacting antibodies. J. Allergy Clin Immunol. 98, 969-978

DISCLAIMER:

ELISA SYSTEMS excludes all representations, warranties, conditions and promises of any kind (express or implied) in relation to the product supplied ("the Product"), including any warranty or conditions in relation to the quality, fitness or suitability of the Product, except for any warranties which, by law, ELISA SYSTEMS cannot exclude. The Buyer assumes all risk and liability for the Product, its use or the fitness of the Product for any purpose.

In any event, ELISA SYSTEMS' liability for breaching any implied warranty or conditions is limited to the replacement of the Product.

Food Allergen Kits available:

• Almond • Beta-Lactoglobulin • Buckwheat • Casein • Crustacean
• Egg • Gluten • Hazelnut • Mustard • Peanut • Sesame • Soy

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