

ATTACHMENT-24



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. This assay is a rapid and reliable test which significantly reduces the time required to screen appropriate food products for the presence of *Soy 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 flour proteins were chosen for the detection of Soy protein residue material for this assay.

Interpretation of Results:

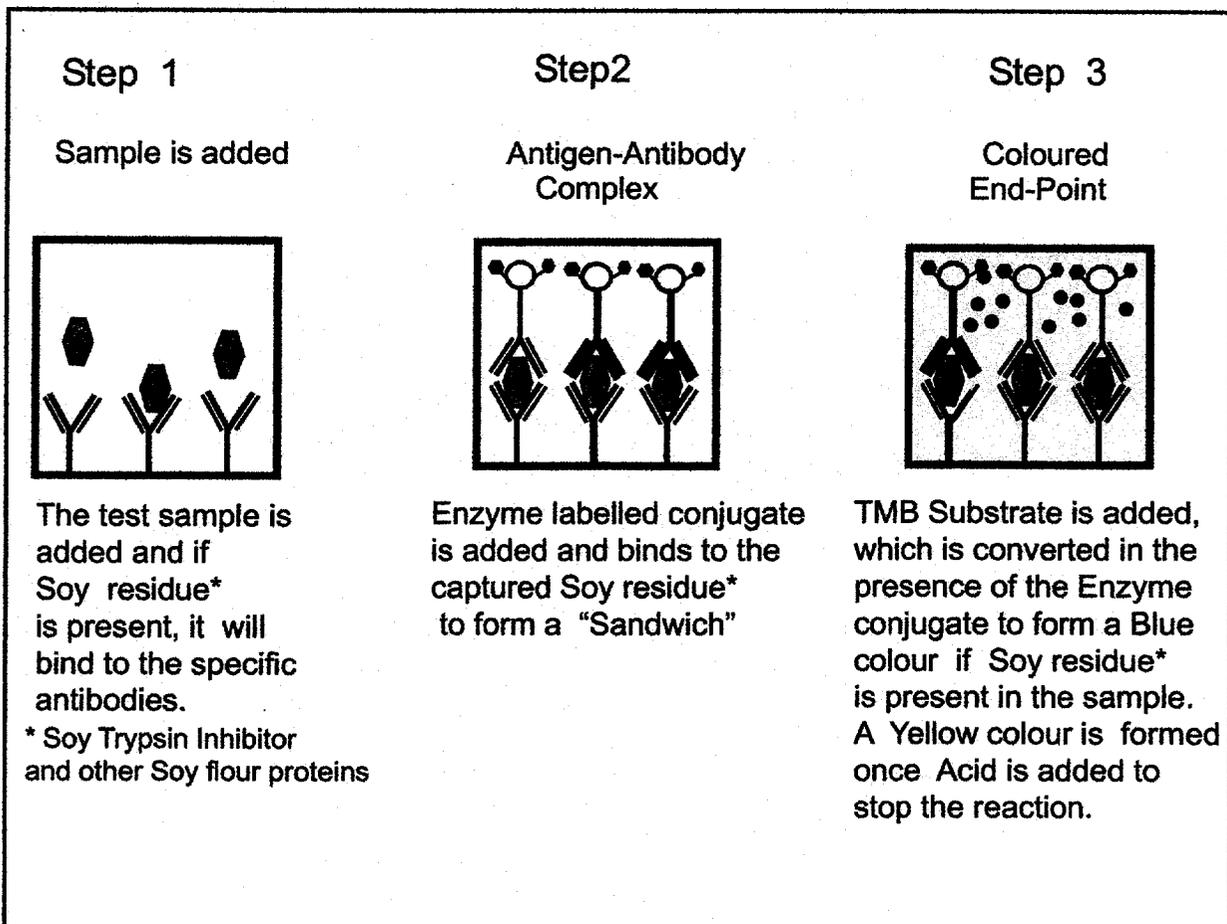
This assay is based on comparison to Soy Flour Protein Concentrations

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 flour protein* antibodies coated onto microwells. After addition of the sample and the enzyme conjugate, 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, based on the detection of Soy Trypsin Inhibitor and other Soy flour proteins. 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 assay is designed for screening purposes.**
A positive result indicates the need to conduct further testing.

How the ELISA SYSTEMS Soy Protein Residue test works:



Reagents Supplied

Test Strips: microwells containing anti-Soy Protein antibodies- 48 wells.

Test strip holder: One (1)

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

Positive Controls:

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 6 ml of Peroxidase conjugated *anti-Soy Protein* polyclonal antibodies with preservative.

Substrate : One (1) bottle containing 10 ml of a stabilized tetramethylbenzidine (TMB).

Wash Buffer concentrate solution (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 10 ml of 1 M 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 medical attention if the irritation persists or is severe.

Additional Materials Required:

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°C. Paper towels. Distilled or Deionized water.

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 (optional). If only a 450nm filter is available, this should be sufficient in most cases.

Please Note: Changes to the Protocol for Swab samples on Page 5

Please note: A special extraction procedure is required for samples consisting of or containing Dark Chocolate or Tannins. Please contact ELISA SYSTEMS or your representative for details of this procedure if you are to test samples containing Dark Chocolate or Tannin.

Precautions

Do not use solutions if they precipitate or become cloudy.

Exception: 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.

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 Extracted samples 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.

All pipette volumes should be \pm 1 microlitre

Do not pour or return unused Enzyme Conjugates and Substrate back into their bottles.

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 from possible allergenic material or reagents from other test kits or previous test runs. This is especially the case when any extraction additives may have been used for specific assay extractions.

Storage Conditions

Reagents, strips and bottled components:

Store between 2 - 8° C. 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

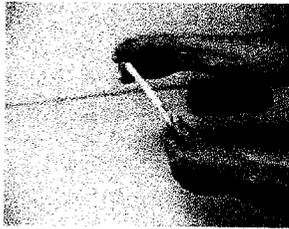
Remove the cap and add contents of one bottle to 475 ml DI water. Transfer contents of diluted wash buffer into a squeeze bottle (small tip bottle). For long term storage, label the storage bottle containing the diluted Wash buffer with the kit lot number and kit expiry date.

Extraction Solution

Remove the cap and add contents of one bottle to 475 ml DI water. Transfer contents of diluted extraction solution into a storage bottle. For long term storage, label the storage bottle containing the diluted Extraction buffer with the kit lot number and kit expiry date.

Food Allergen/Residue Swab sampling Protocol

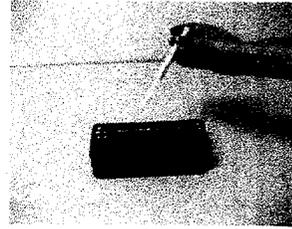
Suggested procedure: May 2005



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.



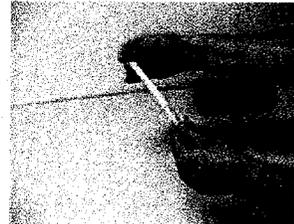
Remove excess moisture from the
swab tip by pressing on the inside
of the swab tube.



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.

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Sample Preparation

A representative sample(s) must be taken from the product.

The sample must be blended/grounded to a fine consistency to provide a homogeneous mixture.

Pre-Warm the Diluted Extraction Solution to 60 °C

The pH of the extracted sample should be in the range of 6.8 to 7.4

Please note: A special extraction procedure is required for samples consisting of or containing Dark Chocolate or Tannins. Please contact ELISA SYSTEMS for details of this procedure if you are to test samples containing Dark Chocolate or Tannin.

FOR SOLID SAMPLES

Weigh out 5 grams of finely ground sample into a suitable blender, a Stomacher Type bag or a similar device. Add 50ml of the pre-warmed diluted Extraction Solution.

A ratio of 1 part sample plus 10 volumes of the prepared Extraction Solution must be used.

Blend or Stomach until the sample is homogenous and only minimal clumps are present.

Complete mixing to remove clumps will help ensure consistent results. 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. Filter the extract through Filter paper (medium fast grade) or similar.

Samples may be centrifuged. The filtrate or supernatant is then collected. Mix well.

This is then the sample to be tested on the kit.

FOR LIQUID SAMPLES

Place 5 ml of sample into a suitable blender, bottle or similar device and add 45ml of the pre-warmed diluted Extraction Solution. ***A ratio of 1 part sample plus 9 volumes of the prepared Extraction Solution must be used for liquid samples.***

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. Filter the extract through Filter paper (medium fast grade) or similar. Samples may be centrifuged. The filtrate or supernatant is then collected.

Mix well. This is then the sample to be tested on the kit.

FOR SWAB SAMPLES (Please Note: Changes from Previous suggested procedures)

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 fresh 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/mixing for one minute every 5 minutes.

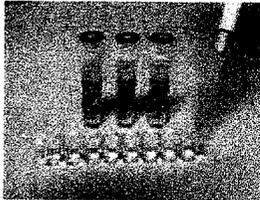
After the completion of the incubation and mixing stage, remove from the water bath and allow to settle. Filtration is normally not required. Decant the extract into a small test tube.

Mix well. This is then 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 should not be used to quantify the absolute amount of allergen proteins, but used as a general indication for monitoring of the levels present.

Food Allergen Residue ELISA Protocol

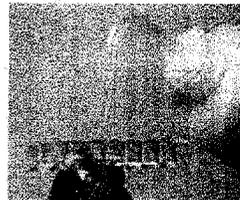
Always check the kit insert for the current test procedure



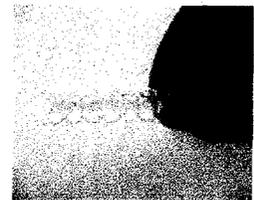
Add 100 microlitres of Controls and Samples to their allocated Antibody coated wells.
Mix all wells for 10 seconds by gentle shaking on a flat surface
Incubate for 30 mins.



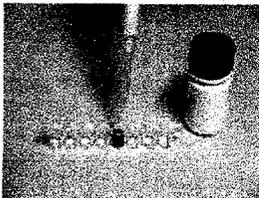
Dump liquid from wells



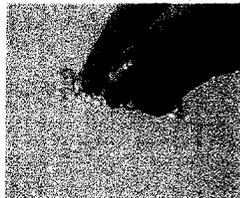
Wash wells thoroughly Five times with wash buffer.



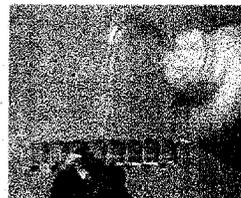
Tap wells firmly onto absorbent paper towel



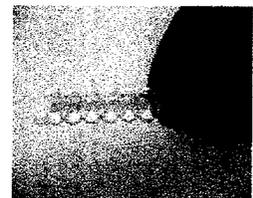
Add 100 microlitres of the Green Conjugate Solution to each well.
Mix all wells for 10 seconds by gentle shaking on a flat surface
Incubate for 30 mins.



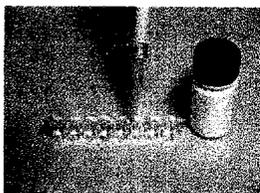
Dump liquid from wells



Wash wells thoroughly Five times with wash buffer.

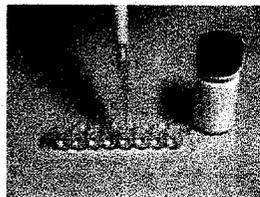


Tap wells firmly onto absorbent paper towel



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 mins.

DO NOT WASH

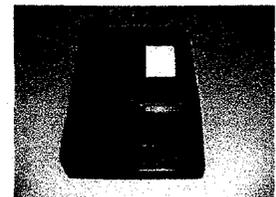


Add 100 microlitres of the Stop Solution to each well.
Mix all wells for 10 seconds by gentle shaking on a flat surface



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

June 2005 v1.4



Test Procedure

Screening Method.

Photocopy the Sample Coding Sheet supplied on Page 11.

Ensure all kit components are at room temperature (20 – 25°C) prior to commencing this assay. At least two of the kit controls must be included each time the assay is run.

Choose the most appropriate Positive control for your screening. This may depend on the sample matrix being tested.

For Screening purposes, use the 2.5ppm control as the Positive Control. (suggested) More Controls may be used if required for determination of levels.

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.

1. Break off the number of wells needed (number of samples plus at least two wells for controls) and place in the strip holder. Use a fine tipped marker pen to place an identification mark on each strip (not on the well bottom) to allow for correct identification of the wells in the strip holder.
Refer to your Sample Coding Sheet for the position of the Samples and the kit controls.
2. Add 100microlitres of the extracted test sample(s) to the appropriate 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 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. After the 15 minutes incubation step for the Substrate, 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 & 620 - 650nm. Zero the reader on air.
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 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 is not thoroughly washed from the well before addition of subsequent reagents.

Test Procedure *Quantitative Screening Method.*

Photocopy the Sample Coding Sheet supplied on Page 11.

Controls for a Standard Curve, must be included each time the assay is run.

Ensure all kit components are at room temperature (20 – 25°C) prior to commencing this assay.

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. All pipette volumes should be \pm 1 microlitre.

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 (minimum of 4 wells including the Negative Control for a standard curve) and place in a separate Control Column in the well holder.
Use a fine tipped marker pen to place an identification mark on each strip (not on the well bottom) to allow for correct identification of the wells in the strip holder.
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 2.5ppm Positive control to well #2 of the Control Column
Add 100 microlitres of the 5.0ppm Positive control to well #3 of the Control Column
Add 100 microlitres of the 10.0ppm Positive control to well #4 of the Control Column
(if required) Add 100 microlitres of the 25.0ppm 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. After the 15 minutes incubation step for the Substrate, 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 & 620 - 650nm. Zero the reader on air.

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 is not thoroughly washed from the well before addition of subsequent reagents.

Interpretation of Results This assay is based on Soy Flour Protein Concentrations

Interpretation is based on the suggested extraction/dilution protocol.

Any sample returning a Positive result should be regarded as a Presumptive result and confirmation or further testing should be performed.

The user should determine the value or readings to be considered as "Not Significant"

Please refer to the Notes on Page 12.

Screening Method

Visual or ELISA Reader

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.

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.

Quantitative Method

ELISA Reader

Zero the ELISA Reader on air. Read all wells using a microstrip reader, preferably with bichromatic filters at 450nm and 620- 650nm. If only a 450nm filter is available, this should be sufficient in most cases.

An Absorbance (OD) reading equal to or greater than the (OD) reading of the chosen Positive control well, indicates the sample contains Soy protein equal to or greater than the control value of Soy protein. If a quantitative curve is prepared, the value obtained indicates an approximation of the level of Soy protein in the sample

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.

Troubleshooting

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												

Assay Name _____

Date Performed _____

Operator _____

Room Temperature _____

Comments _____

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.

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

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

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- (a) the performance of the Product; or
- (b) any fact, matter or thing relating to the Product; or
- (c) any error (whether negligent or in breach of contract or not) in information supplied to the Buyer or a user before or after the date of the Buyer's or user's use of the Product.

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Kits available:

Almond, Beta Lactoglobulin, Casein, Crustacean,
Egg, Hazelnut, Peanut, Sesame, Soy