Beta-Lactoglobulin Residue

Product Code: ESMRDBLG-48

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

For screening for the presence of beta-lactoglobulin residue in food products and environmental samples.

Directions For Use

Intended Use

The ELISA SYSTEMS Beta-Lactoglobulin (BLG) Residue assay is an enzyme-linked immunosorbent assay (ELISA) that may be used to screen food products for the presence of this allergen caused by cross-contamination with Milk and Dairy Products.

This kit can be used to detect whole milk or whey. For dairy products from which whey has been removed (e.g. some cheeses), our Casein kits may be more suitable. Please discuss this with your ELISA SYSTEMS representative.

Background

Beta-Lactoglobulin is the major whey protein in ruminants and pigs. It is not found in the milk of many species.[1] Milk from humans appears to be devoid of Beta-Lactoglobulin.

Human infants can develop an allergenic response to cow milk proteins. Beta-Lactoglobulin is the primary antigenic component that stimulates the immune hypersensitivity response in the infant.[1]

The ELISA SYSTEMS Beta Lactoglobulin ELISA is a rapid and reliable test which significantly reduces the time required to screen food products for the presence of Beta-Lactoglobulin. A conversion table is supplied for expression of results in terms of other milk products.

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 BLG Residue ELISA is a double antibody (sandwich) ELISA utilizing specific anti-Beta-Lactoglobulin antibodies coated onto microwells. After addition of the sample, the Enzyme Conjugate, then the TMB Substrate, a positive reaction (indicating the presence of Beta-Lactoglobulin) 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.

This assay will detect beta-lactoglobulin present in the sample and indicates the presence of milk material. Comparison of the samples with the supplied positive standards allows estimation of beta-lactoglobulin present in the sample.

A conversion table is supplied on page 10 to allow reporting of the results in terms of different milk products.

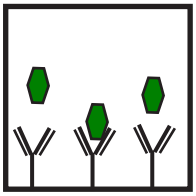
The results of the testing are only applicable to the portion of the sample product tested and to this extent, ELISA SYSTEMS cannot guarantee that milk material is, or is not, present in the untested portions of the sample product.

This 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 Beta-Lactoglobulin Residue test works:

Step 1

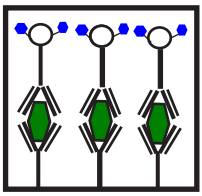
Sample is added



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

Step 2

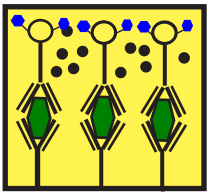
Antigen-Antibody Complex



Enzyme-labelled Conjugate is added and binds to the captured BLG 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 BLG residue is present in the sample.

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

Reagents Supplied

Test Strips: microwells coated with anti-Beta-Lactoglobulin antibodies – 48 wells.
Test strip holder: One (1)

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

Positive Standards: Beta-Lactoglobulin (BLG)
One (1) vial containing 1.7mL of BLG in a buffer to provide a Control value of 0.10 ppm BLG
One (1) vial containing 1.7mL of BLG in a buffer to provide a Control value of 0.25 ppm BLG
One (1) vial containing 1.7mL of BLG in a buffer to provide a Control value of 0.50 ppm BLG
One (1) vial containing 1.7mL of BLG in a buffer to provide a Control value of 1.00 ppm BLG

Enzyme Conjugate:
One (1) bottle containing 7mL of Peroxidase-conjugated anti-Beta-Lactoglobulin polyclonal antibodies with preservative.
Please note - this reagent is light-sensitive. Avoid unnecessary exposure of the reagent to light.

Substrate: One (1) bottle containing 7mL of a stabilized Tetramethylbenzidine (TMB).
Please note - this reagent is light-sensitive. Avoid unnecessary exposure of the reagent to light.

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

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

Stop Solution: One (1) bottle containing 7mL 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. •Clean test tubes or small microtubes for aliquotting the Enzyme Conjugate and Substrate volumes prior to use. •Data record sheets. •Fine-tipped marking-pen. •Laboratory Timer. •Paper towels.
•Plastic wash bottle with a fine tip. •Distilled or Deionized water. •Laboratory Vortex machine. •Laboratory balance capable of measuring at least 2 decimal places.
•Pipettes: 20-200 microlitre; 100-1000 microlitre (optional, for aliquoting reagents); 20-200 microlitre multichannel pipette if using more than two strips per run; disposable tips.
•Water Bath, capable of heating and holding the samples at 60°C during extraction. •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

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

Do not add azides to the samples or any of the reagents. Standards 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 repeatedly from the TMB Substrate and Enzyme Conjugate bottles as this could contaminate these solutions. Determine the required volumes of these reagents and dispense the volumes required into clean test tubes just prior to use. Do not pour or return unused Enzyme Conjugate or TMB 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 - 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 or heat.

Reagent Preparation

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.

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. To ensure consistent results, a suitable blender, food processor, or a similar mixing device should be used to blend or mix the sample until it is homogenous.

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

FOR SOLID SAMPLES

A ratio of 1 part sample plus 10 parts of the prepared Extraction Solution must be used. For most samples, weigh out 5 grams of finely-ground sample into a suitable clean container for extraction purposes, and add 50mL of the working-strength Extraction Solution. If samples are considered fully homogenous and representative of a larger batch, a smaller sample volume may be used, as long as the 1 + 10 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, and shake/mix the sample(s) for one minute every five minutes. Alternatively, if your water bath has a shake function, place the sample(s) into the water bath at 60°C for 15 minutes, and set the water bath on a gentle shake setting, to ensure the samples mix well. 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). Some samples may require centrifugation. Collect the aqueous phase from each sample - 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 5mL of sample into a suitable container, centrifuge tube, or similar device and add 45mL of the working-strength Extraction Solution. If samples are considered fully homogenous 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, and shake/mix the sample(s) for one minute every five minutes. Alternatively, if your water bath has a shake function, place the sample(s) into the water bath at 60°C for 15 minutes, and set the water bath on a gentle shake setting, to ensure the samples mix well. 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). Some samples may require centrifugation. Collect the aqueous phase from each sample - this is the sample to be tested on the kit.

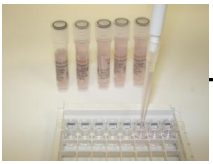
FOR SWAB SAMPLES

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. Pre-moisten 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.

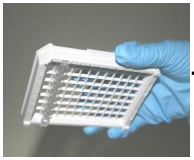
To extract the material, add 1mL of the appropriate, diluted Extraction Buffer to the Swab tube and place the sealed Swab tube into a water bath at 60°C for 15 minutes, with shaking or mixing for one minute every five minutes. Vortexing is recommended. Alternatively, if your water bath has a shake function, place the sample(s) into the water bath at 60°C for 15 minutes, and set the water bath on a gentle shake setting, to ensure the samples mix well. 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). 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.

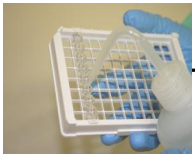
Food Allergen Residue ELISA Protocol



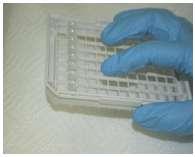
Add 100 microlitres of Standards and Samples to their allocated Antibody-coated wells.
Mix all wells for 10 seconds by gentle shaking on a flat surface.
Incubate for 15 minutes.



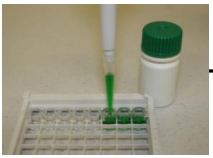
Dump liquid from wells.



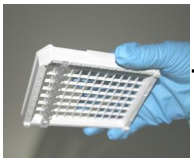
Wash wells thoroughly five times with wash buffer.



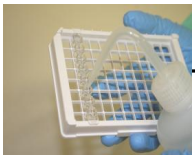
Tap wells firmly onto absorbent paper towel.



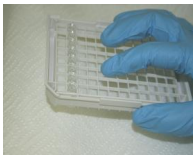
Add 100 microlitres of the Green Conjugate Solution to each well.
Mix all wells for 10 seconds by gentle shaking on a flat surface.
Incubate for 15 minutes.



Dump liquid from wells.



Wash wells thoroughly five times with wash buffer.



Tap wells firmly onto absorbent paper towel.

DO NOT WASH

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 10 minutes.

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 Standards.
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, standards and other reagents, otherwise timing errors may occur.

Record the positions of the standards and samples in the assay. An example Sample Coding Sheet is supplied on Page 11 if required.
Ensure all kit components are at room temperature (20 – 25°C) prior to commencing this assay.
The negative and at least one positive standard (the 0.1 ppm standard is suggested) must be included each time the assay is run. The choice of the positive control may depend on the sample matrix being tested.

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

Calculate the amount of the Enzyme Conjugate and the Substrate Solution required. Determine the number of wells to be used in the assay, multiply by 0.1mL and add about 20% 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. (These reagents are light-sensitive and should be protected from prolonged exposure to light).

1. Break off the number of wells needed (number of samples plus the number of standards) and place in the test strip holder. Refer to your Sample Coding Sheet for the position of the samples and the kit standards. Use a fine-tipped marker pen to place an identification mark on the tab at the end of the 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 correct test well(s) starting in column 1.

3. After all the samples have been added to the wells in accordance with your sample coding sheet, add 100 microlitres of the negative standard followed by 100 microlitres of the selected positive standard(s) to the appropriate wells.

   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 15 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 15 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 10 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 wash cycle consists of:
1. Invert the plate and flick out the contents of each well into a sink or waste container.
2. Use the diluted wash buffer to fill each well to overflowing.
3. Invert the plate and flick out the wash solution.

Repeat steps 2 and 3 until each well has been washed five times.
Invert the wells, and tap out any residual wash solution onto absorbent paper towels.
Alternatively, use an automatic plate washer to aspirate then fill wells five times, then tap onto paper as described above.

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, standards and other reagents, otherwise timing errors may occur.

Record the positions of the standards and samples in the assay. An example Sample Coding Sheet is supplied on Page 11 if required.
Ensure all kit components are at room temperature (20 – 25°C) prior to commencing this assay.
Standards for a Standard Curve must be included each time the assay is run.
Mix the standards thoroughly prior to each use, preferably with a laboratory vortex machine.

Calculate the amount of the Enzyme Conjugate and the Substrate Solution required. Determine the number of wells to be used in the test, multiply by 0.1mL and add about 20% 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. (These reagents are light-sensitive and should be protected from prolonged exposure to light).

1. Break off the number of wells needed for the samples and place in the test strip holder. Break off the number of wells for the standards and place in a separate Control Column in the strip holder. Use a fine-tipped marker pen to place an identification mark on the tab at the end of the 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 standard and each of the positive standards to the appropriate test wells.

   Mix wells by moving strip holder gently sideways for 10 seconds.
   Incubate at room temperature for 15 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 15 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 10 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 wash cycle consists of:
1. Invert the plate and flick out the contents of each well into a sink or waste container.
2. Use the diluted wash buffer to fill each well to overflowing.
3. Invert the plate and flick out the wash solution.

Repeat steps 2 and 3 until each well has been washed five times.
Invert the wells, and tap out any residual wash solution onto absorbent paper towels.
Alternatively, use an automatic plate washer to aspirate then fill wells five times, then tap onto paper as described above.

Interpretation of Results

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

NOTE: The negative standard, 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 standard(s) should be a distinct yellow colour. If there is no yellow colour in the positive standards, the test should be regarded as invalid and should be repeated. If the positive standards again show 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 quantitation, samples should fall in the range of the standards supplied (0.10 - 1.00 ppm BLG). Samples 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

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

Quantitative Method

ELISA Reader

Read all wells using a microstrip reader, preferably with bichromatic filters at 450nm (primary) and 620-650nm (secondary/reference).

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. Quantitative results are best determined in the lower to middle region of the standard curve (0.1 - 0.5ppm).

The lower limit of Quantitation for this assay is the value of the lowest positive standard which is 0.10 ppm Beta Lactoglobulin. Results that indicate a value of greater than 0.05 ppm should be further investigated.

Milk Conversion Tables (Beta-Lactoglobulin Kit)

Equivalent amounts of different milk products that give the same kit reaction. Results are expressed as ppm or mg of allergen detected per kg of sample.

Kit Standard	Beta-Lactoglobulin	Total Milk Protein	Skim Milk Powder	Whole Milk Powder	Whole Milk	Skim Milk
0	0	0	0	0	0	0
0.1	0.1	1.0	3.12	4.0	31	31
0.25	0.25	2.5	7.81	10.0	78	78
0.50	0.50	5.0	15.60	20.0	156	156
1.00	1.00	10.0	31.25	40.0	312	312

Sample Coding Sheet

	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 _____ 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 _____

Quality Control

The use of the kit positive and negative standards allows validation of kit stability. For a valid test, the kit standards 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 standard has substantial colour development.
Correction: Washings were insufficient. Repeat test with more vigorous washings.

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 the addition of subsequent reagents.

Caution: There are many combinations of formulations, additives, processes, treatments, etc, that may affect the food sample and 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 standard 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 milk 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°C.

Reference.

1. Hurley, W.L. “Milk Proteins and Protein Synthesis”in “Lactation Biology”University of Illinois, Urbana-Champaign Http://classes.aces.uiuc.edu/AnSci308/proteinsyn.html

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
• Gliadin • Hazelnut • Lupin • Mustard • Peanut • Sesame • Soy

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January 2014