

Casein Residue

Product Code: ESCASPRD - 48

Microwell ELISA For Laboratory Use Only Store Between 2 - 8^o C

**For screening for the presence of Milk Casein Residue
in Food Products and Environmental Samples**

Directions For Use

Intended Use

The ELISA SYSTEMS Casein Residue assay is an enzyme-linked immunosorbent assay (ELISA) that may be used to screen food products for Casein as an indicator of the presence of milk or milk products.

Background

Casein is the major protein in milk. It accounts for about 80% of total milk proteins. It is used widely in the food industry, along with derivatives and isolates from milk.

Casein is a source of food allergies and must be excluded from the diet of susceptible individuals.

The **ELISA SYSTEMS** Casein Residue ELISA is a rapid test which significantly reduces the time required to screen food products for the presence of Casein.

This assay has been shown to detect Casein from several mammalian species as well as from Bovine milk. It should not be used as an indication of specific Bovine Casein.

The assay expresses results in terms of Skim Milk Powder (non-fat) equivalents. 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 consisting of or containing Polyphenols, including Dark Chocolate, Wine, Fruit Juices, Tannins.



Principle of Procedure

The ELISA SYSTEMS Casein Residue ELISA is a double antibody (sandwich) ELISA utilizing specific anti-Casein antibodies coated onto microwells. After addition of the substrate solution, following the sample and the enzyme conjugate steps, a positive reaction (indicating the presence of Casein) produces a deep 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 Casein* present in the sample and indicates the presence of Milk material. Comparison of the samples with the supplied positive controls allows estimation of Total Skim Milk Powder (non fat) present in the sample. Casein constitutes approximately 27% of Skim Milk Powder mass.

A conversion table is supplied on page 10 to allow reporting of the results in terms of different milk products. For example: a result of 10ppm using the kit control would equate to 2.57 ppm Casein.

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.

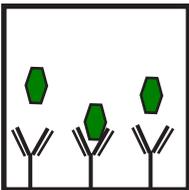
The assay is designed for screening purposes.

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 Casein Residue test works:

Step 1

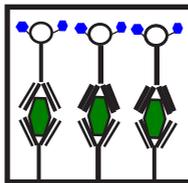
Sample is added



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

Step2

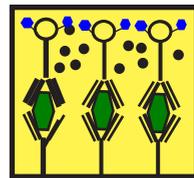
Antigen-Antibody Complex



Enzyme labelled conjugate is added and binds to the captured Casein 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 Casein residue is present in the sample.

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

* Alpha S Casein

Reagents Supplied

Test Strips: microwells containing anti–Bovine Casein 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 Milk Powder in a buffer to provide a Control value of 1.0 ppm

One (1) vial containing 1.7 ml of Milk Powder in a buffer to provide a Control value of 2.5 ppm

One (1) vial containing 1.7 ml of Milk Powder in a buffer to provide a Control value of 5.0 ppm

One (1) vial containing 1.7 ml of Milk Powder in a buffer to provide a Control value of 10.0 ppm

Enzyme Conjugate:

One (1) bottle containing 7 ml of Peroxidase conjugated anti-Bovine Casein polyclonal antibodies with preservative.

Substrate : One (1) bottle containing 7 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 7 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 urgent 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. 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 (optional)

Please note: A special extraction solution is required for samples consisting of or containing Polyphenols, including Dark Chocolate, Wine, Fruit Juices, Tannins.

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.**

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 the 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.

All pipette volumes should be ± 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**



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.

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

Please note: A special extraction solution is required for samples consisting of or containing Polyphenols, including Dark Chocolate, Wine, Fruit Juices, Tannins.

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 homogenous and only minimal clumps are present. Complete mixing to remove clumps so as to 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 and reach room temperature. Filter the extract through Filter paper (medium fast grade) or similar and collect the filtrate. Alternatively, the samples may be centrifuged and the supernatant is 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 homogenous and representative of a larger batch, a smaller sample volume may be used, as long as the 1 : 9 ratio is maintained. 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.

Filter the extract through Filter paper (medium fast grade) or similar and collect the filtrate. Alternatively, the samples may be centrifuged and the supernatant is 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/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. 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 15 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 15 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 10 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

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Test Procedure

Screening Method only.

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°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 1.0 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 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 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 to the appropriate well.
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 & 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 are not thoroughly washed from the well before addition of subsequent reagents.

Test Procedure **Quantitative Screening Method only.**

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.

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, 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 (minimum of 5 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 1.0 ppm Positive control to well #2 of the Control Column
Add 100 microlitres of the 2.5 ppm Positive control to well #3 of the Control Column
Add 100 microlitres of the 5.0 ppm Positive control to well #4 of the Control Column
Add 100 microlitres of the 10.0 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 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 & 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 are not thoroughly washed from the well before addition of subsequent reagents.

Interpretation of Results

This assay is based on comparison to Skim Milk Powder (non fat) concentrations.

Interpretation is based on the suggested extraction/dilution protocol.

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.

For Quantitative assays, the sample should fall in the range of the standards supplied (1 - 10 ppm Skim Milk Powder) 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.

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.

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 Milk material 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.

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

The lower limit of Quantitation for this assay is the value of the lowest Positive Control which is 1.0 ppm Skim Milk Powder.

Milk Conversion Tables (Enhanced Casein 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 Control	Skim Milk Powder	Whole Milk Powder	Total Milk Protein	Casein	Whole Milk	Skim Milk
0	0	0	0	0	0	0
1	1	1.3	0.32	0.26	10	10
2.5	2.5	3.2	0.80	0.64	25	25
5	5	6.4	1.6	1.28	50	50
10	10	12.8	3.2	2.57	100	100

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 _____ Room Temperature _____

Assay Times: Samples and Controls _____ Conjugate _____

Comments: _____

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.

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 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.

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.

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Kits available:

**Almond, Buckwheat, Beta Lactoglobulin, Casein,
Crustacean, Egg, Gluten, Hazelnut, Mustard, Peanut,
Sesame, Soy**