

Total Milk Residue



Food Safety Solutions

Product Code: ESTMLK-48

Microwell ELISA For Laboratory Use Only. Store Between: 2°C

For screening for the presence of milk residue in food products and environmental samples.

Directions For Use

Intended Use

The ELISA SYSTEMS Total Milk Residue assay is an enzyme-linked immunosorbent assay (ELISA) that may be used to screen food products for the presence of milk proteins. The assay detects α S-casein as a marker of casein and β -lactoglobulin as a marker of whey protein.

Background

Milk is a source of food allergies and must be excluded from the diet of susceptible individuals. Milk protein is comprised of casein and whey proteins. Casein and whey protein are present together in whole milk, but may also be present individually in some products.

The ELISA SYSTEMS Total Milk Residue ELISA is a rapid test which detects both casein and whey proteins. It can be used to detect contamination of foods by milk, whey or casein.

This assay has been shown to detect protein from several ruminant species. As well as bovine milk, this assay shows cross-reactivity with goat's and ewe's milk. This assay should not be used as an indicator of specific bovine casein.

The assay expresses results in terms of milk protein 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, Herbs, Spices and Tannins (product code: ESADDSOL).

Principle of Procedure

The ELISA SYSTEMS Total Milk Residue ELISA is a double antibody (sandwich) ELISA, utilizing specific anti-casein and anti- β -lactoglobulin antibodies coated onto microwells. After addition of the sample, the Enzyme Conjugate, then the TMB Substrate, a positive reaction produces a deep blue colour (indicating the presence of milk protein*). 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 milk protein present in the sample and indicates the presence of milk. Comparison of the samples with the supplied positive standards allows the estimation of the concentration of skim milk powder present in the sample.

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

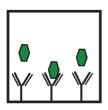
The results of the testing are only applicable to the portion of the sample product tested and to this extent, ELISA SYSTEMS cannot guarantee that milk protein is, or is not, present in the untested portions of the sample product.

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.

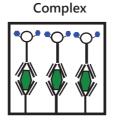
How the ELISA SYSTEMS Total Milk Residue test works:

Step 1Sample is added



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

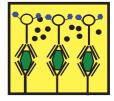
Step 2
Antigen-Antibody



Enzyme-labelled Conjugate is added and binds to the captured milk 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 milk 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-Casein and anti-BLG antibodies – 48 wells.

Test strip holder: One (1)

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

Positive Standards:

Please note - all positive standard values are expressed in Skim Milk Powder equivalents.

One (1) vial containing 1.7mL of Milk Protein in a buffer to provide a Control value of 0.25 ppm

One (1) vial containing 1.7mL of Milk Protein in a buffer to provide a Control value of 0.5 ppm

One (1) vial containing 1.7mL of Milk Protein in a buffer to provide a Control value of 1.0 ppm

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

Enzyme Conjugate:

One (1) bottle containing 7mL of Peroxidase-conjugated anti-milk protein 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): Two (2) bottles containing 25mL each of concentrated wash buffer solution with preservative.

Extraction Solution concentrate (20x): Four (4) 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.

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.

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.

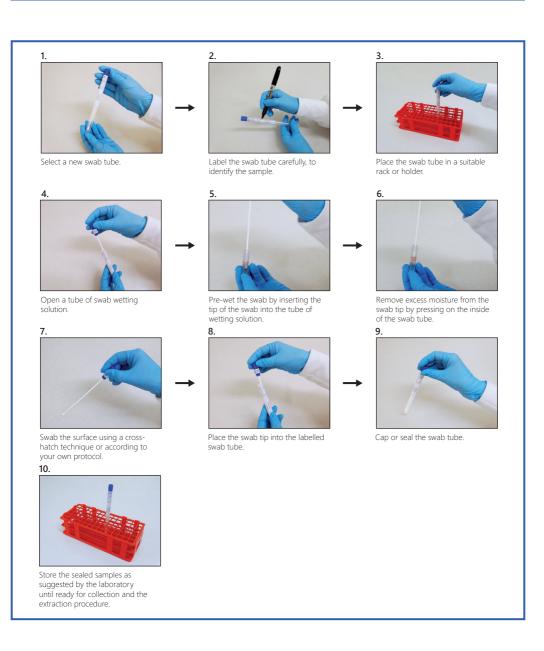
Wash Buffer

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



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 $5.00 \pm 0.05g$ of finely-ground sample into a suitable clean container for extraction purposes, and add 50 ± 1 mL 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 $60^{\circ}\text{C} \pm 5^{\circ}\text{C}$ 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 $60^{\circ}\text{C} \pm 5^{\circ}\text{C}$ 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 5.00 ± 0.05 mL of sample into a suitable container, centrifuge tube, or similar device and add 45 ± 1 mL 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 $60^{\circ}\text{C} \pm 5^{\circ}\text{C}$ 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 $60^{\circ}\text{C} \pm 5^{\circ}\text{C}$ 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.

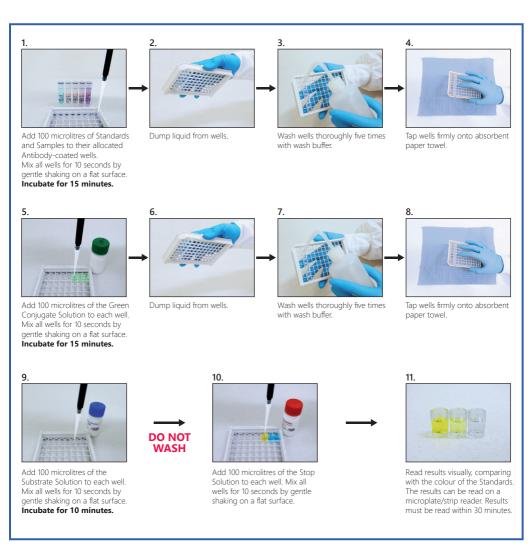
For Swab Samples

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 1.00 ± 0.01 mL of the appropriate, diluted Extraction Buffer to the Swab tube and place the sealed Swab tube into a water bath at 60° C \pm 5° 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 \pm 5° 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





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 $(20 - 25^{\circ}\text{C})$ prior to commencing this assay. **The negative and at least one positive standard (the 0.25 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).

All pipette volumes should be \pm 1 microlitre.

- 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 correctly 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 well.

Mix wells by moving strip holder gently sideways for 10 seconds.

Incubate at room temperature for 15 minutes, then wash.#

 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.#

incubate at room temperature for 13 minutes, then wasn.#

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 $(20 - 25^{\circ}\text{C})$ prior to commencing this assay. **All the kit standards** <u>must be included each time</u> 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 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).

All pipette volumes should be ± 1 microlitre.

- 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. 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 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.#

- 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.#
- 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
- 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 Control Standards. Results are expressed as ppm or mg of allergen detected per kg 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.

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 Food Allergen Risk Management Plan.

Limit of Detection (LOD)

Ideally each testing laboratory should determine the LOD as part of an in-house validation study. If this has not been determined then the lower limit of application of 0.125ppm should be used.

Qualitative Method

Visual or ELISA Reader

Compare the colour or Optical Density (OD) of the sample well(s) 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 residue 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 (0.25 - 1.0ppm).

For quantified results, samples MUST fall into the range of the supplied positive standards (0.25 - 2.5ppm). Do not extrapolate above or below this range. Extracts returning high results can be diluted in the Extraction Solution and retested, but remember to apply the extra dilution factor in concentration calculations.

Milk Conversion Table

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	Total Milk Protein	Skim Milk Powder ^a	Whole Milk ^b
0	0	0	0
0.25	0.25	0.71	7.6
0.50	0.50	1.43	15
1.0	1.0	2.86	30
2.5	2.5	7.14	76

Conversion Factors based on:

a. Skim Milk Powder is 35% protein

b. Whole Milk is 3.3% protein



Sample Coding Sheet

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Quality Control

The use of the kit positive and negative standards allows validation of kit stability. It is strongly recommended that an internal control/check sample of consistent concentration is included with every run. When performing Quantitative analysis, the Standard Curve obtained should closely resemble the shape of the Standard Curve shown by the kit manufacturer.

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 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.

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 人 Almond Milk 人 Beta-Lactoglobulin 人 Buckwheat Casein 人 Crustacean 人 Egg 人 Gluten 人 Hazelnut 人 Hazelnut Milk Lupin 人 Mustard 人 Peanut 人 Sesame 人 Soy 人 Soy Milk 人 Total Milk