Version 24-04/24

User's Manual

Casein ELISA Kit

REF **DEIA289**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Enzyme Immunoassay for the Quantitative Determination of bovine Casein in Food

General Description

Bovine milk belongs to the most important allergenic food ingredients especially for children. Already very low amounts of bovine milk can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, milk allergic persons must strictly avoid the consumption of milk or milk containing food. In particular the presence of hidden milk proteins such as in sausage, cookies, convenience food or beverages represent a critical problem for milk allergic persons. According to EU Directive 2003/89/EG the addition of bovine milk has to be labeled. For the detection of bovine milk in foodstuffs, sensitive detection systems are required. Approximately 80% of bovine milk proteins are caseins which are composed of α-, β- and κ-caseins. So these heat-stable allergens represent the main fraction of bovine milk proteins. The Casein ELISA represents a highly sensitive detection system and is particularly capable of the identification and quantification of bovine casein residues in cookies, bread crumbs, sausage, orange juice, wine, soy products and chocolate.

Principles of Testing

The Casein quantitative test is based on the principle of the enzyme linked immunosorbent assay. An antibody directed against casein is bound on the surface of a microtiter plate. Casein containing samples or standards are given into the wells of the microtiter plate. After 20 minutes incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugated second antibody directed against casein is given into the wells and after 20 minutes of incubation the plate is washed again. A substrate solution is added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of casein is directly proportional to the colour intensity of the test sample.

Reagents And Materials Provided

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with anti-casein antibodies.
2. Casein Standards (0, 0.2, 0.6, 2, 6 ppm of casein): 5 vials with 1.0 mL as 100× concentrate, dyed blue. Dilute 20 µL of standard with 1980 µL pre-diluted extraction and sample dilution buffer to achieve the concentrations named above. Stored at 4°C the diluted standards are stable for at least 24 hours. Note: The concentrations above refer to the 100x diluted standards.
3. Conjugate (anti-casein-peroxidase): 15 mL, dyed red, ready-to-use.
4. Substrate Solution (TMB): 15 mL, ready-to-use.
5. Stop Solution (0.5 M H_2SO_4): 15 mL, ready-touse.

6. Extraction and sample dilution buffer (Carbonate buffer): 2 × 120 mL as 5× concentrate, dyed red. Dilute 1+4 with distilled water. Stored at 4°C the diluted buffer is stable for at least one week. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
7. Washing Solution (PBS + Tween 20): 60 mL as 10× concentrate. Dilute 1+9 with distilled water. Stored at 4°C the diluted buffer is stable for at least 4 weeks. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
8. Plastic bag to store unused microtiter strips.
9. Instruction Manual.

Materials Required But Not Supplied

Instrumentation

1. 100 - 1000 µL micropipets
2. Analytical balance
3. Mortar, mixer
4. Water bath
5. Centrifuge
6. ELISA reader (450 nm)

Reagents

double distilled water

Storage

Stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package. For more detailed information, please download the following document on our website.

Assay Procedure

1. Reagent And Sample Preparation

Due to a high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be cleaned thoroughly before and after each sample. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions.

The following sample preparation should be applied for solid samples:

1) To maximize homogeneity and representativeness of the sample drawing, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.
2) 0.5 g of the homogenized mixture is suspended in 10 mL of pre-diluted extraction and sample dilution buffer. Afterwards the suspension is incubated for 15 min in a preheated water bath at 60°C. To ensure good homogeneity, the samples should be shaken every two minutes.
3) The samples are centrifuged for 10 minutes at 2000 g. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
4) Due to high matrix effects meat and sausage samples should be further diluted 1 + 4 with prediluted

extraction and sample dilution buffer.

5) 100 µL of particle-free solution are applied per well. If the results of a sample are out of the measuring range, further dilution with the prediluted extraction and sample dilution buffer is necessary. The additional dilution has to be considered when calculating the concentration.

The following sample preparation should be applied for liquid samples:

1) 0.5 mL of liquid sample is diluted in 9.5 mL of prediluted extraction and sample dilution buffer. Afterwards the suspension is incubated for 15 min in a preheated water bath at 60° C. To ensure good homogeneity, the samples should be shaken every two minutes. The process is continued at point 3 of solid sample extraction process.

2. Assay Steps

The washing solution is supplied as 10× concentrate and has to be diluted 1+9 with double distilled water before use. In any case the diluted standards should be determined at least twofold. When samples in great numbers are determined, the standards should be pipetted once before the samples and once after the samples. For final interpretation the arithmetic mean is used for calculation. In consideration of GLP and quality control requirements a duplicate measurement of samples is recommended. The procedure is according to the following scheme:

1) Prepare samples as described above.
2) Pipet 100 µL of diluted standards or prepared samples in duplicate into the appropriate wells of the microtiter plate.
3) Incubate for 20 minutes at room temperature.
4) Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances.
5) Pipet 100 µL of conjugate (anti-casein-peroxidase) into each well.
6) Incubate for 20 minutes at room temperature.
7) Wash the plate as outlined in 4.
8) Pipet 100 µL of substrate solution into each well.
9) Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 20 minutes at room temperature.
10) Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue colour will turn yellow upon addition.
11) After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

Calculation

1. Evaluation

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 6 ppm standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Casein (ppm)	% binding of 6 ppm
6	100
2	75
0.6	44
0.2	23
0	8

2. Calculation

The diluted standards are prepared for a direct determination of sample concentrations. The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered. Additional dilution due to meat containing samples or high sample concentration has to be accounted for.

1) Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2) Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ppm on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively the evaluation can be carried out by software. In this case the 4-parameter method should be preferred.
3) Using the mean optical density value for each sample, determine the corresponding concentration of casein in ppm from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed. For calculation of the amount of a corresponding raw product, the casein concentration has to be multiplied with a product specific conversion factor (F).

The following conversion factors have been determined by means of validation experiments:

Whole milk	42
Non fat milk powder (NIST RM1549)	3.6
Whole milk powder (NIST RM8435)	4.9
Caseinate	1.2

Precision

Intra-assay Precision: 5 - 11%

Inter-assay Precision: 8 - 14%

Sensitivity

The limit of detection (LOD) of the Casein test is 0.04 ppm. The limit of quantification (LOQ) of the Casein test is 0.2 ppm. Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

Specificity

For the following foods no cross-reactivity could be detected:

Wheat, Sesame, Rye, Sacharose, Oats, Chicken, Barley, Pork, Corn, Beef, Rice, β-Lactoglobulin, Egg.

The following cross-reactions were determined:

Ewe's milk <1.2%

Goat's milk <1.1%

Linearity

The serial dilution of spiked samples (cookies, bread crumps, chocolate, sausage, soy milk, orange juice and white wine) resulted in a dilution linearity of 80% - 102%.

Recovery

Mean recovery was determined by spiking samples with different amounts of casein:

Cookies 100%

Bread crumps 80%

Chocolate 86%

Sausage 80%

Soy milk 94%

Orange juice 84%

White wine 102%

References

1. Hefle SL, et al. (2004) – Validated sandwich enzyme-linked immunosorbent assay for casein and its application to retail milk-allergic complaint foods. J Food Prot, 67(9):1933-38
2. Patrick W, et al. (2009) – Determination of the bovine food allergen casein in white wines by quantitative indirect ELISA, SDS-Page, Western blot and immunostaining. J Agric Food Chem, 57(18):8399-405
3. Watanabe H et al. (2005)- Study on detection of allergenic substances (egg and milk) in processed meat products and frozen foods. Sho Eis Zas, 46(4):139-47