Casein ELISA Kit

Enzyme Immunoassay for the quantitative determination of bovine Casein in food

Catalog number: ARG80790

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INTRODUCTION

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, and soy products and chocolate.

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantitative determination of bovine Casein in food.

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody specific for Casein is bound onto a pre-coated 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 color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The absorbance is proportional to the concentration of Casein. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP-antibody Conjugate	15 ml (ready to use)	4°C
100X Standards 1-5	5 X 2 ml	4°C
5X Extraction & Sample dilution buffer	2X 120 ml	4°C
10X Wash Buffer	60 ml	4°C
TMB substrate	15 ml (ready to use)	4°C (Protect from light)
STOP solution	15 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or double distilled water
- Mortar, mixer
- Water bath
- Centrifuge
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-Antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer and 5X Extraction & Sample dilution buffer, warm to 37°C for 15 min or until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
- Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- All specimens and standards should be run at the same time, so that all conditions of testing are the same.
- Do not mix components from different batches.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

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

A. 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 & Sample dilution buffer.
3. 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.
4. 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.
5. Due to high matrix effects meat and sausage samples should be further diluted 1 + 4 with pre-diluted Extraction & Sample dilution buffer.
6. 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 pre-diluted Extraction & Sample dilution buffer is necessary. The additional dilution has to be considered when calculating the concentration.

B. The following sample preparation should be applied for liquid samples:

1. 0.5 mL of liquid sample is diluted in 9.5 mL of pre-diluted Extraction & Sample dilution buffer.
2. 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 process is continued at point 4 of solid sample extraction process as above.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 10X Wash Buffer into double distilled water to yield 1X Wash Buffer. (e.g. 60 ml of 10X Wash Buffer + 540 ml of double distilled water) The diluted wash buffer is stable for at least 4 weeks when stored at 4°C.
- 1X Extraction & Sample dilution buffer: Dilute 5X Extraction & Sample dilution buffer into double distilled water to yield 1X Extraction buffer. (e.g. 50 ml of 5X Extraction & Sample dilution buffer + 200 ml of double distilled water) The diluted Extraction & Sample dilution buffer is stable for at least one week when stored at 4°C. If crystals are observed in the Extraction & Sample dilution buffer, warm to 37°C for 15 min or until the crystals are completely dissolved.
- Standards: Dilute 20 µl 100X standards with 1980 µl pre-diluted Extraction & Samples dilution buffer to achieve the concentration as below. The diluted standards are stable for 24 hours at 4°C. The diluted standards have the following concentration: 0, 0.2, 0.6, 2, 6 ppm.

Standard No	Concentration
Standard 1	0 ppm
Standard 2	0.2 ppm
Standard 3	0.6 ppm
Standard 4	2 ppm
Standard 5	6 ppm

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in duplicates. 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.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of diluted standards and prepared samples in duplicate into wells.
3. Incubate for 20 minutes at RT.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl of HRP-Antibody Conjugate into each well. Incubate for 20 minutes at RT.
6. Aspirate and wash well as step 4.
7. Add 100 µl of TMB substrate to each well. Incubate for 20 minutes at room temperature in dark.
8. Add 100 µl of Stop Solution to each well. The blue color will turn yellow upon addition.

9. Read the OD with a microplate reader at **450 nm (reference wavelength 620 nm)** immediately. It is recommended read the absorbance within 30 min after adding the Stop solution.

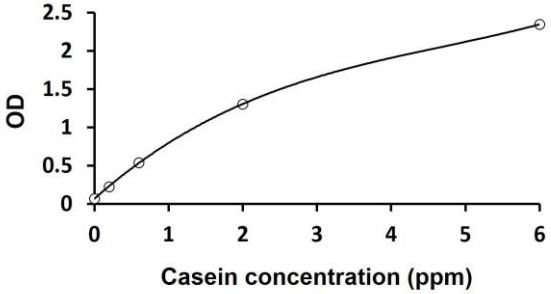
CALCULATION OF RESULTS

1. 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.
2. Calculate the average absorbance values for each set of standards, controls and patient samples.
3. Using semi-log graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
4. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
5. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
6. 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:

Matrix	Conversion factor (F)
Whole milk	42
Skim milk powder (MoniQA MQA 092014)	4.4
Non-fat milk powder (NIST RM1549)	3.6
Whole milk powder (NIST RM8435	4.9
Caseinate	1.2

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

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:

Almond	Cod	Pecan nut	Rye
Barley	Corn	Pine seed	Sacharose
Beef	Egg	Pistachio	Sesame
Brazil nut	Hazelnut	Poppy seed	Shrimps
Buckwheat	Lecithin	Pork	Soy
Chicken	Lima bean	Prawn	Split peas
Chickpea	Oats	Pumpkin seed	ß-Lactoglobulin
Cocoa	Pea	Rice, brown	Walnut
Coconut	Peanut	Rice, white	Wheat

The following cross-reactions were determined:

Ewe's milk	< 1.2%
Goat's milk	< 1.1%

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 5-11% and inter-assay precision was 8-14%.

Recovery

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

Cookies	100%
Bread crumbs	80%
Chocolate	86%
Sausages	80%
Orange juice	84%
White wine	102%
Soy milk	94%

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%.