BioSystems

COD 14113 96 tests
Reagents for the measurement of casein concentration in food samples
Only for *in vitro* use in the laboratory

CASEIN

ELISA

GENERAL INFORMATION

Bovine milk belongs to the most important allergenic food ingredients especially for children. Even 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.

PRINCIPLE OF THE METHOD

The Casein ELISA is an enzyme immunoassay for quantitative analysis of bovine casein residues in cookies, bread crumbs, sausage, orange juice, wine, soy products and chocolate.

Casein in the sample binds to a specific antibody immobilized on the microwell surface. In a second incubation, a peroxidase conjugated second antibody directed against casein binds to surface-bound caseins. Finally, tetramethylbenzidine (TMB) is added to each well as enzyme substrate and, after color development, the enzymatic reaction is stopped with sulfuric acid. The yellow product formed is measured at 450 nm, and it is proportional to the amount of casein present in the sample.

CONTENTS AND COMPOSITION

A. Concentrated Washing Buffer. 60 mL. Phosphate buffered saline.

B. Concentrated Dilution Buffer. 2 x 120 mL. Carbonate buffer. Dyed red.

D. Conjugate. 15 mL. Peroxidase conjugated antibody directed against caseins. Dyed red.

E. Substrate. 15 mL. 3,3',5,5'-tetramethylbenzidine (TMB).

F. Stop Solution. 15 mL. Sulfuric acid 0.5 mol/L.

WARNING: H315: Causes skin irritation. H319: Causes serious eye irritation. P280: Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P332+P313: If skin irritation occurs: Get medical advice/attention. P337+P313: If eye irritation persists: Get medical advice/attention. P362: Take off contaminated clothing and wash before reuse.

M. Microplate. 12 strips of 8 wells each coated with anti-casein antibodies.

S1-S5. Concentrated Casein Standards. 5 x 2.0 mL. Concentration: 0, 0.2, 0.6, 2 and 6 mg/L (ppm). Dyed green.

STORAGE AND STABILITY

Store at 2-8 ºC. Each component is stable until the expiry date marked in the label.

Liquid components are stable once opened until the expiry date marked in the label if they are stored at the recommended temperature, well closed and care is taken to prevent contamination during their use.

Indications of deterioration:

- Reagents: presence of particulate material, turbidity.
- Microplate: rips on the plastic bag, macroscopic defects like scratches on the base of the well.

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines. Any serious incident that might occur in relation to the device shall be reported to BioSystems S.A.

ADDITIONAL MATERIALS REQUIRED (NOT PROVIDED)

- Moist chamber.
- Multitip aspirator or automatic washing equipment for microplates.
- Microplate reader or photometer with microcuvette, with a 450 ±10 nm filter.
- All reagents and materials required for the samples treatment are not provided.

REAGENT PREPARATION

Washing Buffer. Dilute Concentrated Washing Buffer (A) with distilled water in the proportion 1/10. Mix thoroughly. Stable 4 weeks at 2-8ºC.

Dilution Buffer. Dilute Concentrated Dilution Buffer (B) with distilled water in the proportion 1/5. Mix thoroughly. Stable 1 week at 2-8ºC.

Casein Standards. Dilute 20 µL of standard with 1980 µL Dilution Buffer to achieve the concentrations named above. Stable 24 hours at 2-8ºC.

All other reagents are provided ready to use.

Solutions A, B or E may precipitate upon the cold storage. Warm up to 37°C and mix to dissolve before using.

SAMPLE TREATMENT

A homogeneous sample has to be obtained from a representative part of the food.

1. Sample (5 g) is ground and pulverised in a mortar, impact mill, etc. into a fine homogeneous compound (Note 1).
2. Dilute 0.5 g (or 0.5 mL, liquids) of the homogeneous compound with 10 mL (9.5 mL for liquids) of Dilution Buffer and incubate for 15 min in a water bath at 60°C. Shake the suspension every two minutes.
3. Centrifuge the suspension for 10 minutes at 2000 x g. Separate the super-natant from the precipitate completely. Filter if necessary.
4. Meat and sausage samples should be further diluted 1+ 4 with Dilution Buffer.

PROCEDURE

Allow all the reagents and microwells warm up to room temperature. Duplicate determinations are recommended.

1. Open the Casein Microplate package (M) and take out the required amount of wells (Note 2).
2. Pipette 100 µL of the diluted standards (S1-S5) and treated samples into the wells of the plate.
3. Incubate (Note 3) for 20 minutes at room temperature (20-25°C).
4. Aspirate or discard the contents and wash the wells 3 times with 300 µL of Washing Buffer (Note 4).
5. Pipette 100 µL of Conjugate (D) to all wells.
6. Incubate for 20 minutes at room temperature (20-25°C).
7. Aspirate or discard the contents and wash the wells 3 times with 300 µL of Washing Buffer.
8. Pipette 100 µL of Substrate (E) into all wells.
9. Incubate for 20 minutes at room temperature (20-25°C).
10. Pipette 100 µL of Stop Solution (F) into all wells and let stand for 5 minutes at room temperature (Note 5).
11. Read the absorbance of the contents of each well at 450 nm (Note 6). The color is stable for at least 30 minutes.

CALCULATIONS

Plot the absorbance values (mean values of the duplicates) for each standard on the Y axis versus the casein concentrations on the X axis. The concentration of casein in samples is calculated by interpolating the absorbance in the calibration curve (recommended curves: 4-parameter, cubic spline, one site-hyperbola).

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 every new test.

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

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 treatment procedure is already considered.

In case of using the extraction process for meat and sausage samples, the determined concentration has to be multiplied by 5 in order to get the sample concentration.

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:

Sample	factor
Whole milk	42
Skimmed milk powder (MoniQA MQA 092014)	4.4
Skimmed milk powder (NIST RM1549)	3.6
Whole milk powder (NIST RM8435)	4.9
Caseinate	1.2

METROLOGICAL CHARACTERISTICS

- Detection limit: 0.03 ppm.
- Quantification limit: 0.2 ppm. Due to the variety of sample matrices and their influence on the blank, it is recommended that results lower than the quantification limit be treated as negative.
- 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 100 - 127 %.
- Linearity limit: For values higher than 6 ppm dilute the treated sample 1/10 with Dilution Buffer and repeat measurement. This additional dilution has to be considered when calculating the sample concentration.
- Precision: Intra-assay (5-11 %), inter-assay (8-14 %).
- Specificity: Cross-reaction reactivities are < 1.2 % for Ewe's milk and < 1.1 % for Goat's milk. For the following foods no cross-reactivity could be detected:

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

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

Sample	Mean
Cookies	99 %
Bread crumps	84 %
Chocolate	85 %
Sausage	82 %
Soy milk	89 %
Orange juice	87 %
White wine	102 %
Red wine	102 %

NOTES

1. Due to high risk of cross-contamination all used instruments like applicator, mortar, glass vials etc. have to be cleaned thoroughly before and after each sample.
2. Store the unused wells into the plastic bag and reseal it, keeping the desiccant inside.
3. It is recommended to perform all incubations in a moist chamber in order to protect the microplate from evaporation and from light.
4. Manual rinsing or rinsing with automatic plate wash equipment can be performed. Washing Buffer should be completely removed from the wells. Care should be taken not to scratch the inner microwell surface along the procedure.
5. Stop Solution stops the enzyme reaction and must be pipetted into the wells at approximately the same rate as Substrate in step 8.
6. Some microplate readers allow bichromatic readings. In this case, use a secondary wavelength in the range 600-700 nm.

BIBLIOGRAPHY

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