**Casein ELISA Kit**

**Prod. No.: DEIA289**

**Pkg. Size: 96T**

**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.

**PRINCIPLE OF THE TEST**

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 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 100x 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.
5. Stop Solution (0.5 M H₂SO₄): 15 mL, ready-to-use.
6. Extraction and sample dilution buffer (Carbonate buffer): 2 x 120 mL as 100x concentrate, dyed blue. Dilute 1+9 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 10x 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.

**MATERIALS REQUIRED BUT NOT PROVIDED**

**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

**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.
1) Prepare samples as described above.
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 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.
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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. 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:

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
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<tbody>
<tr>
<td>Whole milk</td>
<td>42</td>
</tr>
<tr>
<td>Non fat milk powder (NIST RM1549)</td>
<td>3.6</td>
</tr>
<tr>
<td>Whole milk powder (NIST RM8435)</td>
<td>4.9</td>
</tr>
<tr>
<td>Caseinate</td>
<td>1.2</td>
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</tbody>
</table>

**ASSAY CHARACTERISTICS**

**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.

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

<table>
<thead>
<tr>
<th></th>
<th>Recovery</th>
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<tbody>
<tr>
<td>Cookies</td>
<td>100%</td>
</tr>
<tr>
<td>Bread crumbs</td>
<td>80%</td>
</tr>
<tr>
<td>Chocolate</td>
<td>86%</td>
</tr>
<tr>
<td>Sausage</td>
<td>80%</td>
</tr>
<tr>
<td>Soy milk</td>
<td>94%</td>
</tr>
<tr>
<td>Orange juice</td>
<td>84%</td>
</tr>
<tr>
<td>White wine</td>
<td>102%</td>
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</tbody>
</table>

**Precision**

<table>
<thead>
<tr>
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<th>Value</th>
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<tbody>
<tr>
<td>Intra-assay Precision</td>
<td>5 - 11%</td>
</tr>
<tr>
<td>Inter-assay Precision</td>
<td>8 - 14%</td>
</tr>
</tbody>
</table>

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

**Specificity**

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

- Wheat
- Rye
- Oats
- Barley
- Corn
- Rice
- 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%

**REFERENCES**

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