Bovine milk protein ELISA Kit

Enzyme Immunoassay for the quantitative determination of bovine milk protein in food

Catalog number: ARG80806

Package: 96 wells

ABLE OF CONTENTS

SECTION **Page**

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St.,

Zhubei City, Hsinchu County 302082 , Taiwan

Tel: +886-3-6221320

Fax: +886-3-6221321

Email: info@arigobio.com

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 per-sons 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. ß-Lactoglobulin, the major allergen of whey, represents further 10% of the total protein

The Milk ELISA represents a highly sensitive detection system for milk proteins based on NIST 1549 reference material. The test is likewise capable of the quantification of casein and ß-lactoglobulin residues in food and is validated for cookies, bread crumbs, sausage, orange juice, wine, soy products and chocolate.

PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody mixture is bound on the surface of a microtiter plate. Milk protein containing samples or standards are given in-to 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 mixture directed against Milk proteins 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 yellow color is measured at 450 nm. The concentration of Milk protein is directly proportional to the color intensity of the test sample.

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
Standards 0-4 (0, 0.4, 1.4, 4, 10 ppm)	5 X 1 ml	4°C
5x Extraction and sample dilution buffer	2 X 120 ml	4°C
10x Wash Buffer	60 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 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, Extraction Buffer and Sample diluent buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- 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. Bovine milk protein adhere very strongly to different surfaces. In certain cases they can resist a common dishwasher cleaning. 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 all kinds of 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. 1 g of the homogenized mixture is suspended in 20 mL of pre-diluted extraction 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 2500 g. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
4. 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 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:

0.5 mL of liquid sample is diluted in 9.5 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. The process is continued at point 3 of solid sample extraction process.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.
- 1X Extraction and Sample diluent buffer: Dilute 5X Extraction and Sample diluent buffer into distilled water to yield 1X.
- Standard: Dilute 1:100 with 1X Extraction and Sample diluent buffer.
- Sample: Due to high matrix effects meat and sausage samples should be further diluted 1 + 4 with prediluted extraction and sample dilution buffer.

ASSAY PROCEDURE

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

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 standards and 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 (350 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.

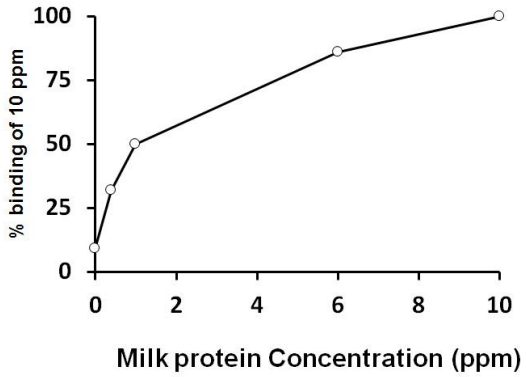
6. Aspirate and wash well as step 4.
7. Add 100 µl of TMB mixture to each well. Incubate for 20 minutes at room temperature in dark.
8. Add 100 µl of Stop Solution to each well.
9. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.
2. The 4 curves for 4 targets can be generated from OD readings of high concentration standard and low concentration standard mixture. The standard curves might not be perfectly straight. To obtain more accurate results, more dilution points can be used when generating standard curves.
3. 4 Parameter Logistics is the preferred method for the result calculation. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure.

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 Milk test is 0.05 ppm of milk protein.

Validation experiments with common matrices resulted in the following LODs [ppm]:

Soy milk	0.13
Orange juice	0.10
White wine	0.03
Bread crumbs	0.08
Cookies	0.16
Chocolate	0.10
Sausage	0.18

The limit of quantification (LOQ) of the Milk test is 0.4 ppm of milk protein.

Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

Specificity

The following cross reactions were determined:

Egg	Sesame	Almond
Wheat	Mustard	Cocoa
Rye	Lupin	Beef
Barley	Celery	Pork
Oats	Peanut	Chicken
Rice	Hazelnut	Cod
Corn	Pistachio	
Soy	Walnut	

The following cross-reactions were determined:

Ewe's milk	0.94%
Goat's milk	0.01%

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 8-10% and the CV value of inter-assay precision was 10-17%

Recovery

Cookies	102%
Bread crumbs	110%
Chocolate	99%
Sausage	88%
Soy milk	79%
Orange juice	106%
White wine	122%