



EuroProxima

# Milk Fraud/Bovine ELISA



*A 96-well microtiter plate immunoassay for the detection of raw and heat-treated cow's and buffalo's milk in the milk of other species and sources*

## Milk Fraud/Bovine ELISA

*An innovative test for the demonstration of milk fraud, based on a cow and buffalo specific sequence on the  $\kappa$ -casein protein.*

In cooperation with the RIKILT (Wageningen, the Netherlands) EuroProxima has developed a competitive enzyme immunoassay for the demonstration of fraudulent mixing of cow and buffalo milk in the higher priced milk of other species (goat, sheep) and sources (soy).

A strong point of this assay is that the part of the  $\kappa$ -casein detected with the monoclonal antibody is heat stable. This means that the ELISA can be used for raw milk as well as heat treated cow and buffalo milk.

The Milk Fraud/Bovine ELISA is based on a monoclonal antibody directed against an epitope of 5 amino acids on the  $\kappa$ -casein molecule. This epitope is unique for  $\kappa$ -casein of bovine species such as cow and buffalo. The wells of the microtiter plate are coated with bovine  $\kappa$ -casein. The monoclonal antibody is conjugated with the enzyme HRP. The binding of the conjugated antibody to the  $\kappa$ -casein on the plate is inhibited by free bovine  $\kappa$ -casein in the milk sample. Ready to use standards for quantification of the cow and buffalo milk content in the sample are provided in the ELISA kit. The % inhibition (fraudulent protein) is measured after addition of substrate. The total time for measuring 40 samples in duplicate (96 wells) is less than 2 hours.

The measuring range for cow and buffalo milk in milk from other species or sources runs from 0.25% up to 50%.

### Bovine milk fraud

The Food and Agriculture Organization (FAO) of the United Nations estimated that 85% of all milk worldwide is produced from cows (1). Aside from cows, many kinds of livestock provide milk used for dairy products. These animals include buffalo, goat, sheep, camel, donkey, horse, reindeer and yak.

The first four respectively produced about 11, 2, 1.4 and 0.2% of all milk worldwide (1).

Due to the differences in production costs, the consumer prices of milk vary a lot and can be up to 17 times higher compared to cow's milk. This encourages fraudulent mixing for economic benefits which is illegal and a risk for consumers with cow's milk allergy or intolerance. The vast majority of children with persistent cow's milk allergy are as well allergic for buffalo's milk (2) and consumption should be avoided as well.

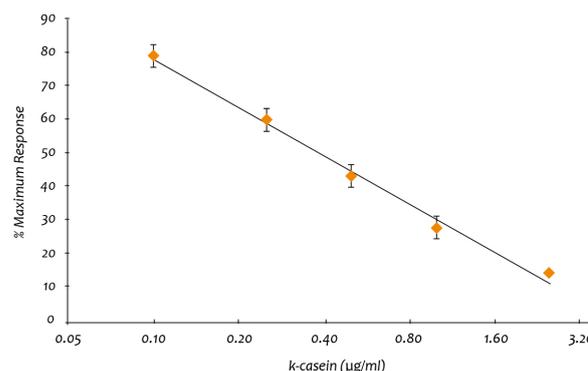
Similar illegal handlings can be expected with the more expensive grain milks (from barley, oat, rice and spelt), legume milks (from lupine, pea, peanut and soy), nut milks (from almond, cashew, hazelnut and walnut) and seed milks (from hemp, quinoa, sesame seed, sunflower seed and coconut).

### References

1. Gerosa S, Skoet J (2012). Milk availability – Trends in production and demand and medium-term outlook. ESA Working paper no. 12-01, February 2012.
2. Sheehan, WJ, Gardynski A, Phipatanakul W. (2009) Skin testing with water buffalo's milk in children with cow's milk allergy. *Pediatr. Asthma Allergy Immunol.* 22(3), 121-125.

### Assay characteristics

#### Calibration curve

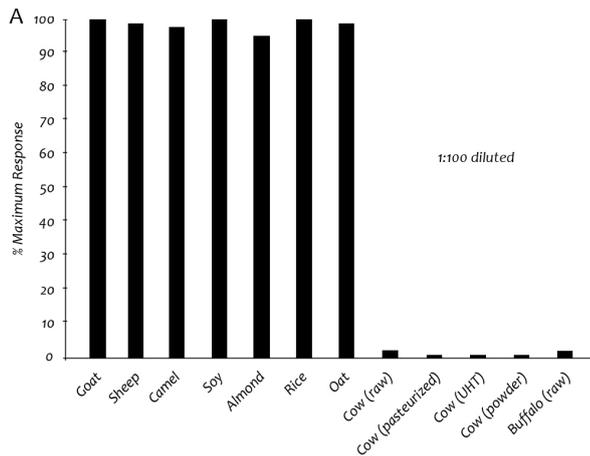


The ELISA uses ready to use calibration standard solutions of bovine  $\kappa$ -casein (0, 0.1, 0.25, 0.5, 1 and 2.5  $\mu\text{g/ml}$ ) and the limit of detection is 0.05  $\mu\text{g/ml}$ .

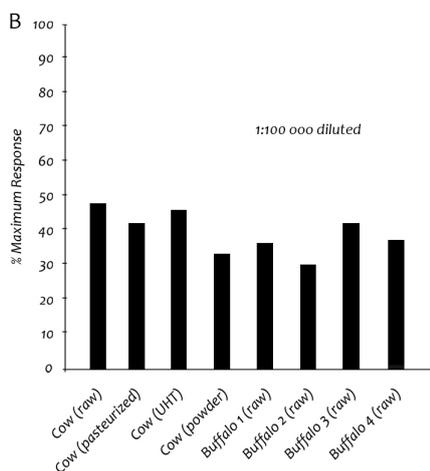
## Specificity

The applied Mab recognises a 5 amino acid-containing epitope on the glycomacropeptide part of bovine  $\kappa$ -casein which is absent from the  $\kappa$ -casein of goat, sheep, horse, donkey, camel, etc. but present on  $\kappa$ -casein of cow and buffalo.

The specificity of the test is shown in table A where the response of milk from different sources is presented.



Maximum inhibition is obtained for cow's and buffalo's milk. No inhibition is seen with milk from goat, sheep, camel, soy, almond, rice and oats, which proves that the ELISA can be applied for the detection of cow's and buffalo's milk in milk of these sources.

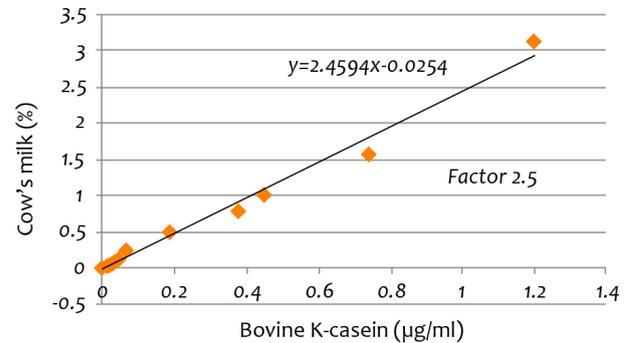


The responses obtained after analysing 1:100 000 diluted cow's and buffalo's milk samples (B) show that small response differences are observed between raw and pasteurized milk but with higher inhibitions for the heat-treated milk samples. This proves that the ELISA suits for the detection of heat-treated cow's milk.

## Sensitivity

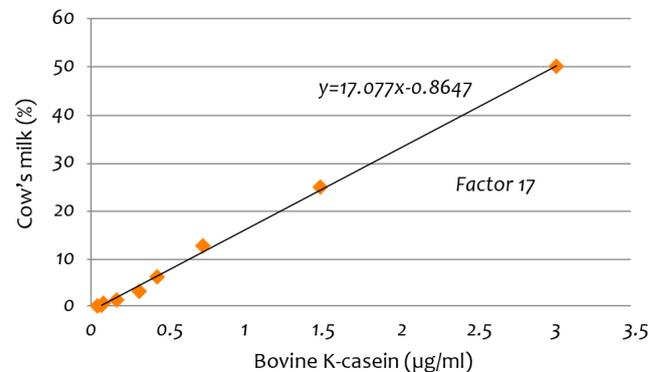
As an example an assay was performed in which cow milk was detected in goat milk samples.

*Milk samples 1:100 diluted:*



The calculation factor for the percentages of bovine's milk and the detected amounts of bovine's  $\kappa$ -casein in 1:100 diluted samples can be read from the linear part of the curve ranging from 0.25 to 2.5% bovine's milk with a slope of 2.5 (1 µg/ml of  $\kappa$ -casein compares with 2.5% of bovine's milk). The decision level (DL) is set at the lowest calibration standard of 0.1 µg/ml which compares with 0.25% bovine's milk.

*Milk samples 1:1000 diluted:*



For quantitative interpretations above 2.5% of bovine's milk, milk samples are analysed after 1:1000 dilutions which results in a linear measurement range from 2% up to 50% bovine's milk.

## Ordering information:

For ordering the Milk Fraud/Bovine ELISA kit, please use catalogue code 5171BKCM



# EuroProxima immunoassays

## Contaminants and Residues

### Beta-Agonists

Beta-Agonist ELISA  
Beta-Agonist Fast ELISA  
Clenbuterol ELISA  
Ractopamine ELISA

### Fungicide

Malachite Green ELISA

### Anthelmintics

Ivermectin ELISA  
Moxidectin ELISA

### Anabolic steroids

Diethylstilbestrol (DES) ELISA  
Ethinylestradiol ELISA  
MedroxyProgesteron Acetate ELISA  
Methyltestosterone ELISA  
Nortestosterone ELISA  
Progesterone ELISA  
Stanozolol ELISA  
Trenbolone ELISA  
Zeranol ELISA

### Corticosteroids

Corticosteroid ELISA  
Triamcinolone ELISA

### Tranquilizers

Azaperone-Azaperol ELISA  
Carazolol ELISA  
Promazine (Generic) ELISA

### Chloramphenicol

Chloramphenicol ELISA  
Chloramphenicol Fast ELISA  
Florfenicol NEW

### Nitroimidazoles

Dimetridazole ELISA

## Tetracyclines

Tetracycline ELISA

## Antimicrobial Growth Promoters

Bacitracin ELISA  
Tylosin ELISA  
Virginiamycin ELISA

## Aminoglycosides

Gentamicin ELISA  
Neomycin ELISA  
Streptomycin ELISA

## Sulfonamides

Dapsone NEW  
Multi-screening Sulfonamides ELISA  
Multi-screening Sulfonamides II ELISA  
Sulfadiazine ELISA  
Sulfamethazine ELISA  
Sulfaquinoxaline ELISA

## Fluoroquinolones

Enrofloxacin ELISA  
Flumequine ELISA  
Fluoroquinolones (Generic) ELISA  
Fluoroquinolones II ELISA

## Nitrofurans

AHD ELISA  
AMOZ ELISA  
AOZ ELISA  
SEM ELISA

## Coccidiostats

Diclazuril ELISA  
Ionophore ELISA

## Shellfish Toxins

Domoic Acid ELISA  
Okadaic Acid ELISA  
Saxitoxin ELISA

## Mycotoxins

Aflatoxin B1 ELISA  
Aflatoxin B1 sensitive ELISA  
Aflatoxin M1 ELISA  
Aflatoxin Total ELISA  
Deoxynivalenol (DON) ELISA  
Fumonisin ELISA  
Ochratoxin A ELISA  
T-2 toxin ELISA  
Zearalenone ELISA

## Mycotoxins Flow Through Rapid Tests

Aflatoxin B1 FTR test  
Aflatoxin total FTR test  
Deoxynivalenol (DON) Gold FTR test  
Ochratoxin A FTR test  
Ochratoxin A in wine FTR test  
Zearalenone Gold FTR test

## Bisphenol A

Bisphenol A (BPA) ELISA NEW

## Meat speciation kits

RAW meat species Kits  
COOKED meat species Kits  
MELISA-TEK® Meat species Kits

## Milk proteins

Bovine Lactoferrin (bLF) ELISA  
Milk Fraud/Bovine ELISA NEW  
Milk Fraud/Whey ELISA NEW

## Immunoaffinity chromatography

Anabolic steroids  
Beta-Agonists  
Corticosteroids  
Antibiotics

## Celiac disease

Gluten-Tec® ELISA

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