Bovine Lactoferrin (bLF)

A 96-well microtiter plate immunoassay for the quantification of bLF in milk and milk products
Bovine Lactoferrin a Novel Food Ingredient

On 2 March 2009, TNO on behalf of the company Friesland Campina submitted a request under article 4 of the Novel Food Regulation (EC) nr. 258/97 to place on the market “bLF” as a novel food ingredient.

The EFSA panel on Dietetic Products, Nutrition and Allergies notes that Lactoferrin is a normal constituent of human milk and the intended consumption of bovine Lactoferrin (bLF) as specified in the application is within the levels of human lactoferrin consumed in breast milk by infants and is safe under the proposed uses and use levels. (EFSA Journal 2012)

Lactoferrin is a 77kDa glycoprotein and member of the transferrin family, a group of proteins capable of binding and transferring Fe3+ ions. Lactoferrin was first isolated from bovine milk (Sorensen, 1939) and was demonstrated in human milk in 1960 (Groves, 1960). Lactoferrin consists of a single chain polypeptide of about 700 amino acids. The sequence homology between Human lactoferrin and bLF is nearly 70%.

Lactoferrin is considered to be part of the innate immune system and due to its strategic position on the mucosal surface of mammary glands it represents a first anti microbial and anti viral defense system. Bacteria require iron for growth and Lactoferrin can inhibit bacteria by chelating iron. Next to this antimicrobial activity Lactoferrin is reported to be an antioxidant that may support the proliferation, differentiation and activation of immune cells and strengthen the immune response. Lactoferrin plays a role in tumor growth that might be related to apoptosis. Lactoferrin stimulates osteoblast proliferation into osteocytes and reduces apoptosis of osteoblasts. The same effect was recorded in chondrocytes. Lactoferrin (L. Adlerova, 2008).

Bovine Lactoferrin is used in meat preservation. It protects fresh beef against different types of pathogens. In the US bLF is permitted at levels of 65 mg/kg for preservation (AS Naidu, 2002). In fish farming bLF is used as diet ingredient up to a concentration of 600 mg/kg (AMM El-Ashram, 2008).

Lactoferrin is found in mucosal secretions, urine and plasma. The highest concentration of human lactoferrin is found in milk and colostrum (7g/L). (DA Rodriguez, 2005). In bovine milk the lactoferrin concentration varies from 0.05 to 0.5 g/L in normal milk. (JB Cheng, 2008). In mastitis milk the concentration is up to 2 g/L, in colostrum 7 g/L (TSuji, 1990).

The source for the production of bLF is skimmed cow milk. According to the EFSA application of Friesland Campina raw cow milk is heated at 50 C and separated in skimmed milk and cream and then the skimmed milk is pasteurized at 72 C. Thereafter the lactoferrin protein is isolated via ion exchange and ultra filtration steps. Ultimately the bLF is dried by means of spraying.

Novel Food

After the European Food Safety Authority approved bLF as novel food ingredient in 2012 the demand for bLF has exploded due to the need for safe baby and infant feed in China and other developing markets. The proposed levels by EFSA are presented in table 1.

The bLF Elisa is a perfect tool to quantify the concentration of Lactoferrin during production and control of the end products by producers as well as controlling agencies.

The table below presents the maximum levels of use of Bovine Lactoferrin as stated in the European Commission Implementing Decision of 22 November 2012.

<table>
<thead>
<tr>
<th>Food group</th>
<th>Maximum use levels of bLF</th>
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<tbody>
<tr>
<td>Infant formulae</td>
<td>100 mg/100 ml</td>
</tr>
<tr>
<td>Foods on dairy basis</td>
<td>200 mg/100 g</td>
</tr>
<tr>
<td>Processed cereal food</td>
<td>670 mg/100 g</td>
</tr>
<tr>
<td>Foods for special medical purposes</td>
<td>Up to 3 g/day</td>
</tr>
<tr>
<td>Beverages based on milk</td>
<td>200 mg/100 g</td>
</tr>
<tr>
<td>Powdered drink mixes based on milk</td>
<td>330 mg/100 g</td>
</tr>
<tr>
<td>Beverages based on fermented milk</td>
<td>50 mg/100 g</td>
</tr>
<tr>
<td>Non-alcoholic drinks</td>
<td>120 mg/100 g</td>
</tr>
<tr>
<td>Products based on yoghurt</td>
<td>80 mg/100 g</td>
</tr>
<tr>
<td>Products based on cheese</td>
<td>2000 mg/100 g</td>
</tr>
<tr>
<td>Ice cream</td>
<td>130 mg/100 g</td>
</tr>
<tr>
<td>Cakes and pastries</td>
<td>1000 mg/100 g</td>
</tr>
<tr>
<td>Candies</td>
<td>750 mg/100 g</td>
</tr>
<tr>
<td>Chewing gum</td>
<td>3000 mg/100 g</td>
</tr>
</tbody>
</table>
World market

In 2012, the total world market for bLF was 185,000 kg/year, worth around € 120 million and is prospected to grow to 260,000 kg/year in 2017 (www.scoop.co.nz/newww/business.html).

Principle of the bLF ELISA

This bLF-ELISA is a competitive immunoassay in a 96-well microtiter plate (12 strips, 8 wells each) with secondary antibody (anti-rabbit)-coated wells. The rabbit anti-bLF, bLF-horseradish peroxidase (bLF-HRP) and standard solutions or samples are added to the wells. Free bLF from standard/samples and bLF-HRP conjugate compete for the specific anti-bLF antibodies bound to the secondary antibodies in the wells. After an incubation step of 1 hour, the non-bound reagents are removed in a washing step. The amount of bound bLF-HRP is visualized by adding a substrate/chromogen solution (H2O2/TMB) which transforms the colourless chromogen into a coloured product.

Specifications

The illustrations show the main characteristics of the Lactoferrin kit. The recovery in different milk matrices was determined at a spike level of 50 µg/ml. The limit of quantification (CCβ) in baby milk powder is 10 µg/ml

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc. (µg/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby milk powder</td>
<td>44.7</td>
<td>89.4</td>
</tr>
<tr>
<td>Semi skimmed chocolate milk</td>
<td>43.8</td>
<td>87.6</td>
</tr>
<tr>
<td>Strawberry drink</td>
<td>60.0</td>
<td>120.0</td>
</tr>
<tr>
<td>Chocolate milk</td>
<td>58.3</td>
<td>116.6</td>
</tr>
<tr>
<td>Soy milk (UHT)</td>
<td>57.9</td>
<td>115.8</td>
</tr>
<tr>
<td>Cow’s whole milk</td>
<td>44.1</td>
<td>88.2</td>
</tr>
</tbody>
</table>

References


Ordering information

For ordering the bLF-ELISA kit, please use cat. code 5091LFER
Contaminants and Residues

**Beta-agonists**
- Beta-Agonist
- Beta-Agonist Fast
- Clenbuterol
- Ractopamine

**Fungicide**
- Malachite Green

**Anthelmintics**
- Ivermectin
- Moxidectin

**Anabolic steroids**
- Diethylstilbestrol (DES)
- Ethynylestradiol
- Medroxyprogesterone Acetate
- Methyltestosterone
- Nortestosterone
- Progesterone
- Stanozolol
- Trenbolone
- Zeranol

**Corticosteroids**
- Corticosteroid
- Triamcinolone

**Tranquilizers**
- Azaperone-Azaperol
- Carazolol
- Promazine (Generic)

**Antimicrobial Growth Promotors**
- Bacitracin
- Tylosin
- Virginiamycin
- Erythromycin

**Shellfish Toxins**
- Domoic Acid
- Okadaic Acid
- Saxitoxin

**Coccidiostats**
- Diclazuril
- Ionophore

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

**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)

**Antibiotics**
- Amphenicoles
- Chloramphenicol
- Chloramphenicol Fast
- Florfenicol

**Aminoglycosides**
- Gentamicin
- Neomycin
- Streptomycin

**Sulfonamides**
- Dapsone
- Multi-screening Sulfonamides
- Multi-screening Sulfonamides II
- Sulfamethazine

**Nitroimidazoles**
- Dimetridazole

**Tetracyclines**
- Tetacycline
- Oxytetracycline

**Fluoroquinolones**
- Enrofloxacin
- Flumequine
- Fluoroquinolones (Generic)
- Fluoroquinolones II

**Nitrofurans**
- AHD
- AMOZ
- AOZ
- SEM

**Beta-Lactams**
- Penicillin

**Meat speciation kits**
- RAW meat species Kits
- COOKED meat species Kits
- MELISA-TEK® Meat species Kits

**Milk proteins**
- Bovine Lactoferrin (bLF)
- Milk Fraud/Bovine
- Milk Fraud/Whey

**Immunoaffinity chromatography**
- Anabolic steroids
- Beta-Agonists
- Corticosteroids
- Antibiotics

**Celiac disease**
- Gluten-Tec®

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**EuroProxima immunoassays**

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