

bovine Lactoferrin (bLF) ELISA



*A 96-well microtiter plate immunoassay
for the quantification of bLF in milk
and milk products*



EuroProxima

Bovine lactoferrin (bLF) activities

bLF plays a key role in the defence mechanisms of the mammary gland of lactating animals. Bacteria require iron for growth, and **bLF** can inhibit bacteria by chelating iron under certain conditions. In addition, **bLF** may directly kill certain bacterial strains or may weaken bacterial resistance by adhesion to the surface of bacteria. Next to this antimicrobial activity, **bLF** is reported to be a natural antioxidant with numerous other functions, including anti-viral, anti-fungal, anti-inflammatory and anti-cancer activities (1,2).

bLF concentrations in milk

The milk **bLF** concentration varies from <0.05 to 0.5 g/L in normal milk (3), is higher in mastitis milk (up to 2 g/l (4)) and goes up to 7 g/L in colostrum (5) and is, as such, a helpful **indicator for the stage of lactation, mastitis and intramammary infection** in dairy cows.

Novel food

Since November 2012, the European Food Safety Authority (EFSA) approved **bLF** produced by FrieslandCampina as “novel food” (6) meaning that it is safe to be used as an ingredient in a variety of foods throughout the European Union, including infant nutrition, at the proposed use levels (see Table). The intended level to be added to milk and milk products is 2 g/kg and highest levels are proposed for sports nutrition (up to 40 g/kg) which is respectively 10 and 200 times higher compared to the normal level in milk.

Bovine lactoferrin (bLF) activities

Food group (6)	bLF (g/kg)
Infant nutrition (<1 year)	1
Infant nutrition – “growing up” milk	2
Foods children aged 1-3 years	6.67
Medical nutrition (liquid + powdered)	1.25
Medical nutrition - tube feeds	0.5
Medical nutrition - others	8
Sports nutrition (liquid + powdered)	3.03
Sports nutrition (bars + other foods)	40
Non-alcoholic drinks	1.2
Cakes and pastries	10
Cheese products	20
Milk and milk products	2
Cold snacks	12
Small candy and candy bars	7.5

Food preservation

bLF is also used in meat preservation. It has been shown to protect fresh beef against more than 30 different types of pathogenic bacteria, including *Salmonella* and *Campylobacter* (7). In the U.S.A., **bLF** is permitted at levels of 65 mg/kg in beef.

bLF added to a traditional Indian milk product (paneer), in levels up to 20 mg/kg, increased the shelf life of the product significantly (8). The ELISA has the potential of being used in these areas as well.

Other applications

bLF is also used in fish farming in which diets containing up to 600 mg **bLF**/kg had a significant positive effect on survival (9). **bLF** is also used in oral hygiene. The ELISA has the potential of being used in these areas as well.

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/neww/business.html).

References

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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.

The reaction is stopped by adding sulfuric acid and the colour intensity is measured photometrically at 450 nm. The optical density is inversely proportional to the **bLF** concentration in the sample.

The ELISA kit contains all reagents to perform the assay. Reagents for sample preparation are not included in the kit.



Easy sample preparation

Milk powder. Add 9 ml of water to 1 g of powder and homogenise for 10 min.

Milk (chocolate milk, flavoured milk, fortified milk, coffee creamer and melted ice-cream).

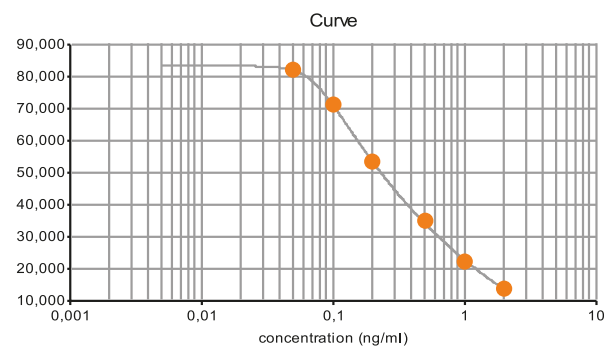
- Vortex the sample for 30 sec.
- Diluted the sample 1:1 with 100% methanol (i.e. 0.2 ml sample + 0.2 ml methanol).
- Vortex for 30 sec.
- Further dilute the sample (1:100) with dilution buffer (10 µl + 990 µl buffer).
- Vortex for 30 sec.

Fast assay protocol

- Pipet 50 µl of each standard and sample solution (in duplicate) into the wells.
- Add 25 µl **bLF**-HRP.
- Add 25 µl anti-**bLF**, shake for a few sec.
- Incubate 1 hour in the dark at RT.
- Discard the solution and wash 3 times.
- Add 100 µl substrate solution.
- Incubate for 30 min in the dark at RT.
- Add 100 µl stop solution, shake for a few sec.
- Read the absorbance values at 450 nm.

Good sensitivity

Example of a calibration curve of the **bLF** ELISA, ranging from 0.05 to 2 µg/ml.



For **bLF** concentrations in the samples, the calculated concentrations have to be multiplied by the dilution factor of 200. This results in a measurement range of 0.01 to 0.4 g/L which complies for levels found in normal milk. With higher sample dilutions higher concentrations can be quantified.

Ordering information

For ordering the **bLF**-ELISA kit, please use cat. code 5091LFFER.

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