Analytical Services

LACTOFERRIN

GENERAL INFORMATION

Introduction: Lactoferrin is an iron-binding antimicrobial protein present in milk and mucosal secretions. It is present at low concentrations in milk throughout lactation and is elevated in milk during infection and in colostrum.

The ELISA assay can be used to estimate lactoferrin concentration in milk from healthy and infected bovine mammary gland. In healthy animals lactoferrin concentration ranges from 20-600 mg/ml. Concentrations over 3000 mg/l have been detected (Hagiwara *et. al.*, 2003).

SAMPLE COLLECTION, PREPARATION AND STORAGE

Type: Milk

Collection: Milk can be stored at 4°C without and with preservative (0.05% bronopol or 0.16% potassium dichromate) for up to 4 days

Preparation: Skim milk is prepared by centrifugation and diluted 1/50 into Tris buffer (50 mM Tris pH 8.0, 0.14 M NaCl, 0.5% BSA, 0.05% Tween20) prior to storage

Storage: Diluted samples are frozen at -80°C long-term

ASSAY

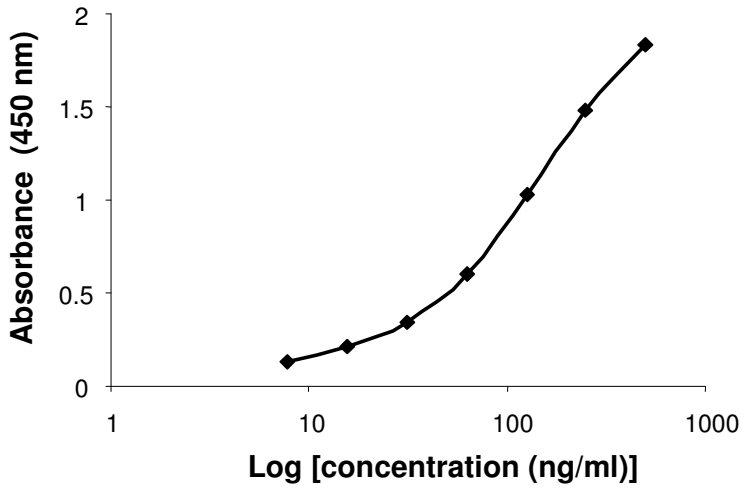
Method:

Sandwich ELISA using polyclonal antibodies against the whole bovine lactoferrin molecule (Bethyl Laboratories Inc., Montgomery, TX) and New Zealand dairy industry lactoferrin standard (gift of Dr. K. Palmano, Fonterra, Palmerston North).

Procedure is essentially that described for the Bovine Lactoferrin ELISA quantification kit (www.bethyl.com). The absorbance is measured using Bio-Rad microplate reader, model 3550 (Bio-Rad, Hercules, CA) and the data is analysed using Flexi 3.04 (Upsdell, 1994).

Sensitivity: 20-4,000 mg/l
Range of Assay: 8-500 ng/ml

Typical Standard Curve:



Quality Assurance:

- Controls – Three controls are included on every plate to determine between plate variability and to monitor the assay
- Interplate repeats – Two samples are repeat between plates to determine variability for samples being analysed

	High 480 mg/l*	Medium 360 mg/l	Low 120 mg/l	Interplate
Coefficient of variation	13%	15%	15%	15%

*Skim milk

REFERENCES

S. Hagiwara, K. Kawai, A. Anri and H. Nagahata (2003) Lactoferrin Concentrations in Milk from Normal and Subclinical Mastitic Cows. *J. Vet. Med. Sci.* 63: 319-323

M.P. Upsdell 1994 Bayesian smoothers as an extension of nonlinear regression. *The New Zealand Statistician* 29: 66-81

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