QUANTITATIVE DETERMINATION OF HUMAN LACTOFERRIN

NEW PRODUCT

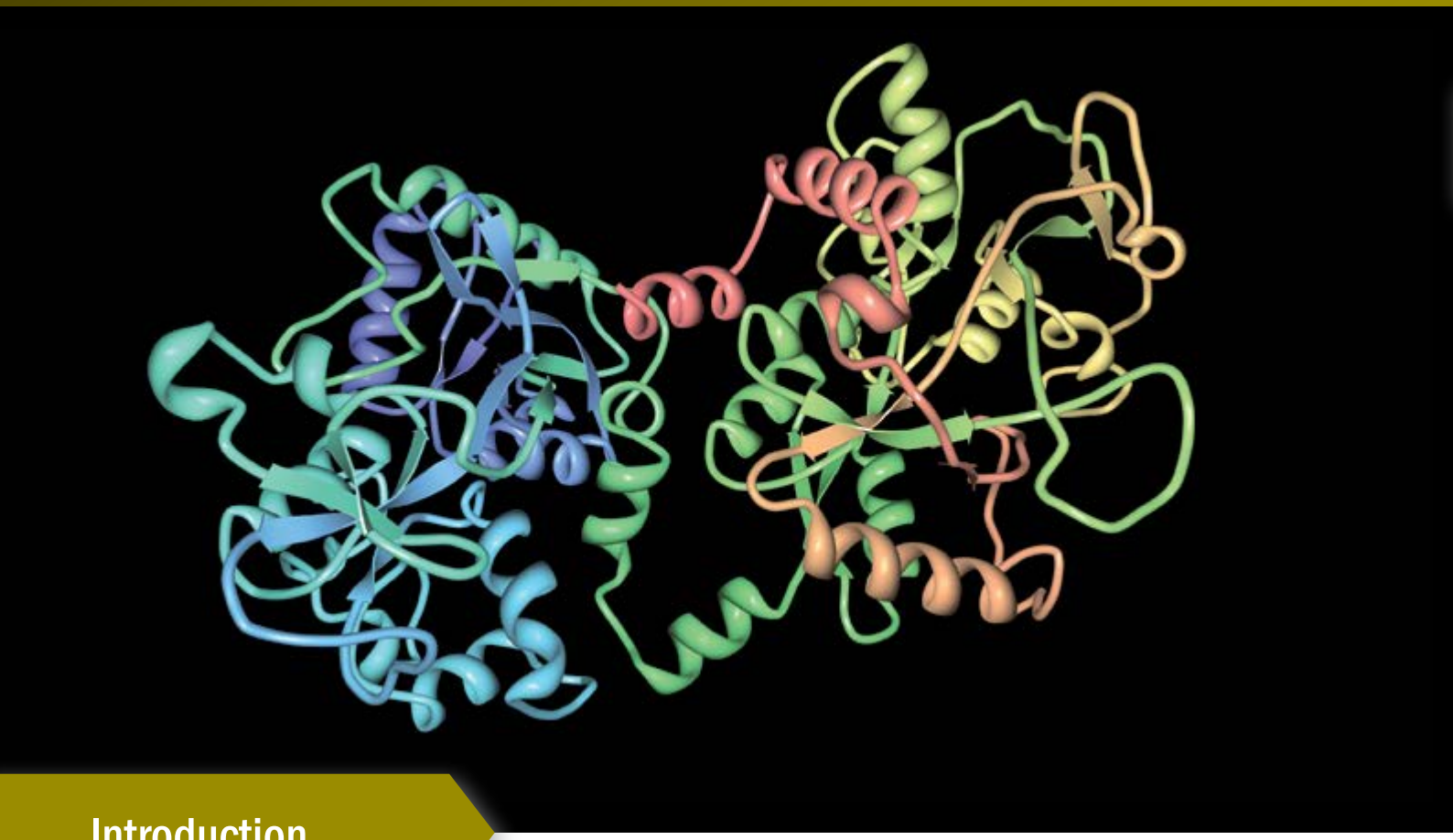
Human Lactoferrin ELISA

- High sensitivity (1.1 ng/ml)
- Excellent analytical characteristics
- Validated for human serum samples and plasma (EDTA) samples, bronchoalveolar lavage fluid (BALF), cerebrospinal fluid (CSF), urine, breast milk, saliva and stool extract

IMMUNE RESPONSE, INFECTION AND INFLAMMATION
INFLAMMATORY BOWEL DISEASE
ENERGY METABOLISM AND BODY WEIGHT REGULATION
LIPOPROTEIN METABOLISM
BONE AND CARTILAGE METABOLISM · ONCOLOGY

BioVendor
Research and Diagnostic Products

HUMAN LACTOFERRIN ELISA



Introduction

Lactoferrin is 703-amino acid iron-binding glycoprotein that belongs to the transferrin family. Lactoferrin capability of binding iron is two times higher than that of transferrin. Two ferric ions can be bound by one lactoferrin molecule. There are three forms of lactoferrin according to its iron saturation: apolactoferrin (iron free), monomeric lactoferrin (one ferric ion), and hololactoferrin (binds two Fe3+ ions). The tertiary structure of hololactoferrin and apolactoferrin is different. [4, 5]

Lactoferrin was originally isolated from breast milk and subsequently was identified in secretions from exocrine glands and in specific granules of neutrophils. Neutrophils after degranulation are main source of lactoferrin in blood plasma. Lactoferrin has been found in most mucosal secretions such as uterine fluid, saliva, bile, pancreatic juice, small intestine secretions, nasal secretion, and tears. [4, 13] Lactoferrin is also present in urine and fecal samples, though levels in these samples are relatively low. The kidney produces lactoferrin in a highly ordered manner but only a minor fraction of the protein is secreted into urine. [6]

The biological properties of lactoferrin are mediated by specific receptors on the surface of target cells and can be found, for example, on mucosal epithelial cells, hepatocytes, monocytes, macrophages, polymorphonuclear leukocytes, lymphocytes, thrombocytes, fibroblasts, and on some bacteria. Lactoferrin possesses various biological functions including its roles in iron metabolism, cell proliferation and differentiation, and antibacterial, antiviral, and antiparasitic activity. Many of these functions do not appear to be connected with its iron binding ability. During most inflammatory reactions lactoferrin concentration increases in all biological fluids, and several authors classify lactoferrin as an acute-phase protein. However, the relationship between its concentration and physiological or pathological effects on body functions is not yet well characterized. [1, 2, 9, 11, 12] Lactoferrin has even been reported to inhibit the development of experimental metastases in mice. [3] Lactoferrin has also been identified as a potent anabolic factor affecting osteocytes, where it induces osteoblast proliferation, survival, differentiation, reduces apoptosis of osteoblasts by 50-70% and inhibits osteoclast formation. [8, 15] The influence of lactoferrin on lipid metabolism was discovered through animal studies. Oral administration of bovine lactoferrin reduced plasma cholesterol levels and retarded hepatic lipid accumulation in mice and decreased serum TAG to 72% of the control level in rats. [7, 10] Another study of abdominally obese men and women showed that ingestion of lactoferrin reduced visceral fat.[14] Fecal lactoferrin level has investigated for its use as a non-invasive marker in the distinction of inflammatory bowel disease (IBD) and non-inflammatory condition. [16]

QUANTITATIVE DETERMINATION OF HUMAN LACTOFERRIN

BioVendor Human Lactoferrin ELISA (RD194334200R)

Intended use

The RD194334200R Human Lactoferrin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human lactoferrin.

- It is intended for research use only
- The total assay time is less than 3.5 hours
- The kit measures lactoferrin in serum, plasma (EDTA), bronchoalveolar lavage fluid (BALF), cerebrospinal fluid (CSF), urine, breast milk, saliva and stool extract
- Special Dilution Buffer is needed for measurement of human lactoferrin in stool extract and is not included. If necessary, for protocol for preparing of stool extract and other details please contact us at info@biovendor.com
- Assay format is 96 wells
- Standard is native protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

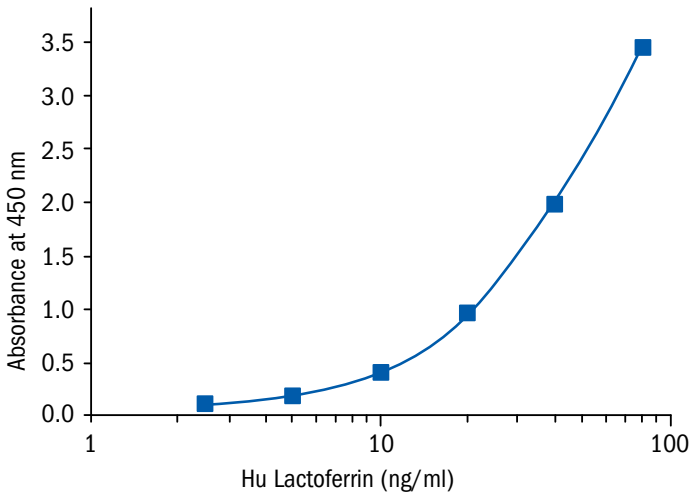
Test principle

In the BioVendor Human Lactoferrin ELISA, standards and samples are incubated in microplate wells pre-coated with monoclonal anti-human lactoferrin antibody. After 60 minutes incubation at 37°C and washing, biotin labelled second monoclonal anti-human lactoferrin antibody is added and incubated for 60 minutes with captured lactoferrin. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation at 37°C and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of lactoferrin. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

Clinical application

- Immune response, infection and inflammation
- Inflammatory bowel disease
- Energy metabolism and body weight regulation
- Lipoprotein metabolism
- Bone and cartilage metabolism
- Oncology

HUMAN LACTOFERRIN ELISA CAT. NO.: RD194334200R	
Assay format	Sandwich ELISA, Biotin-labelled antibody, 96 wells/kit
Samples	Serum, plasma (EDTA), BALF, CSF, urine, breast milk, saliva, stool
Standards	2.5 to 80 ng/ml
Limit of detection	1.1 ng/ml



Precision

Intra-assay (Within-Run) (n=8)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
Serum 1	359.0	11.6	3.2
Serum 2	645.9	21.9	3.4

Inter-assay (Run-to-Run) (n=6)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
Serum 1	345.3	21.5	6.2
Serum 2	731.7	33.2	4.5

Spiking recovery

Samples were spiked with different amounts of human lactoferrin and assayed.

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
Serum 1	324.0	-	-
	416.6	449.0	92.8
	526.0	574.0	91.6
	786.9	824.0	95.5
Serum 2	352.3	-	-
	559.7	552.3	101.3
	739.4	752.3	98.3
	1153.9	1152.3	100.1
EDTA plasma	673.2	-	-
	771.7	798.2	96.7
	1002.5	923.2	108.6
	1222.4	1173.2	104.2
BALF	158.1	-	-
	289.6	258.1	112.2
	380.5	358.1	106.2
	533.7	558.1	95.6
CSF	18.9	-	-
	37.8	33.9	111.5
	48.9	48.9	100.0
	75.1	78.8	95.3
Urine	39.6	-	-
	61.0	54.6	111.7
	67.4	69.6	96.8
	104.3	99.6	104.7

Sample	Observed (µg/g)	Expected (µg/g))	Recovery O/E (%)
Stool	1.6	-	-
	2.2	2.4	91.7
	2.8	3.1	90.3
	4.0	4.6	87.0

Linearity

Samples were serially diluted with Dilution Buffer and assayed.

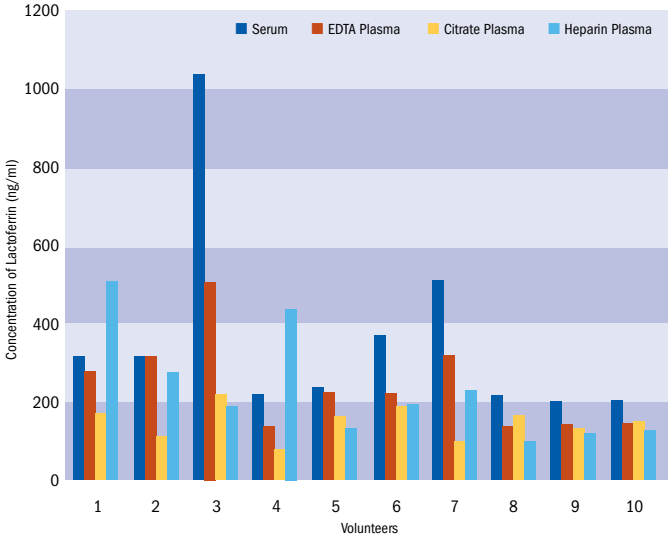
Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
Serum 1	-	748.1	-	-
	2x	357.8	374.0	95.6
	4x	173.5	187.0	92.8
	8x	92.3	93.5	98.7
Serum 1	-	1129.3	-	-
	2x	520.6	564.7	92.2
	4x	261.7	282.3	92.7
	8x	135.0	141.2	95.6
EDTA plasma	-	948.7	-	-
	2x	465.3	474.4	98.1
	4x	228.2	237.2	96.2
	8x	116.5	118.6	98.3
BALF	-	527.3	-	-
	2x	246.0	263.7	93.3
	4x	113.8	131.8	86.3
	8x	56.1	65.9	85.1
CSF	-	37.8	-	-
	2x	19.3	18.9	102.2
	4x	9.1	9.5	96.0
	8x	4.7	4.7	100.3
Urine	-	169.1	-	-
	2x	80.7	84.6	95.5
	4x	40.0	42.3	94.6
	8x	22.2	21.1	105.0

Sample	Dilution	Observed (µg/g)	Expected (µg/g)	Recovery O/E (%)
Breast milk	-	536.9	-	-
	2x	251.5	268.5	93.7
	4x	134.5	134.2	100.2
	8x	65.3	67.1	97.3
Saliva	-	26.8	-	-
	2x	12.6	13.4	94.0
	4x	6.7	6.7	100.0
	8x	3.3	3.4	97.1
Stool	-	18.4	-	-
	2x	9.0	9.2	97.8
	4x	4.2	4.6	91.3
	8x	2.2	2.3	95.7

HUMAN LACTOFERRIN ELISA

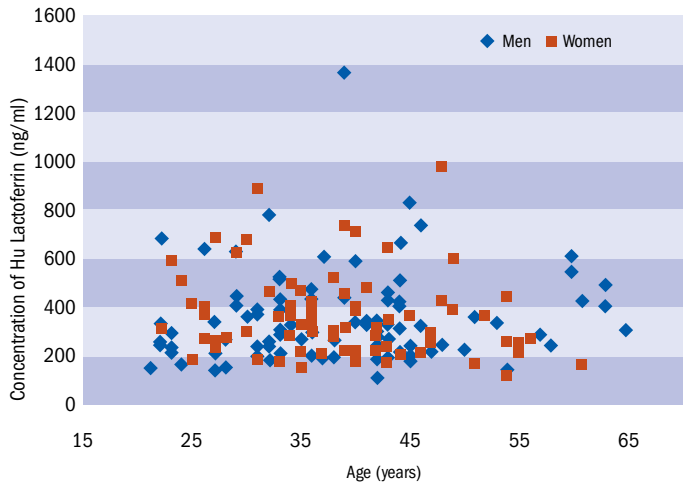
Effect of sample matrix

Heparin, citrate and EDTA plasmas were compared to respective serum samples from the same 10 individuals. However, we observed low correlation among serum and plasma (citrate and heparin) lactoferrin values. Results are shown below:



Preliminary Population Data

The following results were obtained when serum samples from 166 unselected donors (93 men + 73 women) 21-65 years old were assayed with the Biovendor Human Lactoferrin ELISA in our laboratory.



Age and Sex Dependent Distribution of Hu Lactoferrin

Sex	Age (years)	n	Mean Lactoferrin (ng/ml)	Median Lactoferrin (ng/ml)	SD Lactoferrin (ng/ml))	Min. Lactoferrin (ng/ml)	Max. Lactoferrin (ng/ml)
Men	21-29	19	303.6	245.6	170.0	129.0	679.3
	30-39	29	376.8	319.2	233.8	172.7	1368.2
	40-49	31	342.5	314.4	169.0	94.7	824.1
	50-65	14	377.5	368.8	138.4	134.5	606.6
Women	22-29	13	385.2	356.2	156.3	172.0	677.5
	30-39	28	370.5	338.7	169.4	137.4	886.4
	40-49	23	372.9	333.9	194.5	157.6	974.7
	50-61	9	239.7	244.0	96.7	105.1	431.5

Summary of protocol

- Reconstitute Master Standard and prepare set of Standards
- Dilute samples
- Add 100 µl Standards and samples
- Incubate at 37°C for 1 hour without shaking
- Wash plate 5 times
- Add 100 µl Biotin Labelled Antibody
- Incubate at 37°C for 1 hour without shaking
- Wash plate 5 times
- Add 100 µl Streptavidin-HRP Conjugate
- Incubate at 37°C for 30 minutes without shaking
- Wash plate 5 times
- Add 100 µl Substrate Solution
- Incubate at RT for 10 min
- Add 100 µl stop solution
- Read absorbance and calculate results

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