# abcam

### Product datasheet

## Human Lactoferrin ELISA Kit ab200015

Urine

Serum

Cell culture media

Hep Plasma

Recombinant SimpleStep ELISA

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Overview					
Product name	Human Lactoferrin ELISA Kit				
Detection method	Colorimetric				
Precision					Intra-assay
	Sample	n	Mean	SD	CV%
	Human Serum	8			5.1%
					Inter-assay
	Sample	n	Mean	SD	CV%
	Human Serum	3			5.4%
Sample type	Saliva, Milk, Urine, Serum, Cell cu	ulture media	a, Hep Plasma, EDT	A Plasma, C	t plasma
Assay type	Sandwich (quantitative)				
Sensitivity	48.8 pg/ml				
Range	156.3 pg/ml - 10000 pg/ml				
Recovery				S	ample specific recovery
	Sample type	Ave	rage %	Range	
	Saliva	120		113% - 1	26%
	Milk	109		99% - 11	8%

99

102

86

106

99% - 100%

100% - 105%

86% - 86%

105% - 110%

Sample type	Average %	Range
EDTA Plasma	108	105% - 110%
Cit plasma	110	109% - 111%

Assay time

Assay duration

**Species reactivity** 

Product overview

As of October 11 2019, Human Lactoferrin ELISA kit has been re-developed. We have identified new recombinant monoclonal antibodies to provide improved performance and consistency.

Human Lactoferrin ELISA Kit (ab200015) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of Lactoferrin protein in edta plasma, hep plasma, milk, saliva, serum, urine, cell culture media, and cit plasma. It uses our proprietary SimpleStep ELISA® technology. Quantitate Human Lactoferrin with 48.8 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (<u>ab203359</u>) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

#### ASSAY SPECIFICITY

1h 30m

One step assay

Reacts with: Human Does not react with: Cow

This kit recognizes native human Lactoferrin protein in serum, plasma, milk, urine, saliva, and cell culture supernatant samples only.

Cell and tissue extract samples have not been tested with this kit.

#### **CROSS REACTIVITY**

Recombinant human Transferrin protein was prepared at within the working range of the Lactoferrin standard curve and assayed for cross reactivity. No cross-reactivity was observed.

#### INTERFERENCE

Recombinant human Transferrin protein was prepared at 50 ng/mL and 2 ng/mL and assayed for interference in the presence 2 ng/mL human Lactoferrin. No Interference was observed.

#### SPECIES REACTIVITY

This kit recognizes human Lactoferrin protein.

Other species reactivity was determined by measuring 0.5% serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species: Mouse, Rat, Cow, Monkey

Transferrins are iron binding transport proteins which can bind two  $Fe^{3+}$  ions in association with the binding of an anion, usually bicarbonate. Lactoferrin is a member of the transferrin family and is a major iron-binding and multifunctional protein found in exocrine fluids such as breast milk and mucosal secretions. High levels of Lactoferrin are found in saliva and tears, intermediate levels in serum and plasma, and low levels in urine. The canonical sequence of Lactoferrin (known as isoform 1) is 710 amino acids in length and runs at 78.2 kDa. Isoform Delta-Lactoferrin (or DeltaLF) is missing amino acids 1 - 44 of the canonical sequence. Mouse and rat Lactoferrin are 71% and 62% identical to human Lactoferrin, respectively.

Lactoferrin possesses antibacterial, antiviral, anti-inflammatory, and immunoregulatory properties. The presence of Lactoferrin in neutrophils and the release of Lactoferrin during inflammation suggest that Lactoferrin is involved in phagocytic killing and immune responses. Additionally, Lactoferrin mediates bacteriostasis through its ability to sequester free iron and inhibit microbial growth. Lactoferrin also directs bactericidal properties leading to the release of lipopolysaccharides from the bacterial outer membrane. Furthermore, Lactoferrin may play a role in the regulation of bone growth because it has anabolic, differentiating, and anti-apoptotic effects on osteoblasts and can also inhibit osteoclastogenesis. Salivary Lactoferrin plays an important role in protecting the oral cavity against several oral pathogens including Streptococcus mutans. Additionally, increased levels of Lactoferrin in fecal samples is used as a marker of active inflammatory bowel disease (IBD).

Platform

Notes

Microplate (12 x 8 well strips)

#### **Properties**

**Storage instructions** Store at +4°C. Please refer to protocols.

Components	1 x 96 tests	10 x 96 tests
10X Human Lactoferrin Capture Antibody	1 x 600µl	1 x 6000µl
10X Human Lactoferrin Detector Antibody	1 x 600µl	1 x 6000µl

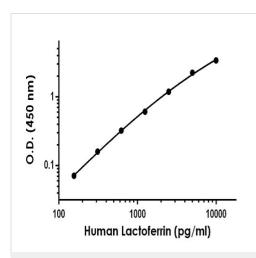
Components	1 x 96 tests	10 x 96 tests
10X Wash Buffer PT (ab206977)	1 x 20ml	1 x 200ml
Antibody Diluent 5BI	1 x 6ml	10 x 6ml
Human Lactoferrin Lyophilized Purified Protein	2 vials	2 x 10 vials
Plate Seals	1 unit	1 x 10 units
Sample Diluent NS (ab193972)	1 x 50ml	2 x 250ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit	1 x 10 units
Stop Solution	1 x 12ml	1 x 120ml
TMB Development Solution	1 x 12ml	1 x 120ml
Function Transferrins are iron binding transpo	ort proteins which can bind two Fe	e(3+) ions in association with

Transferrins are iron binding transport proteins which can bind two Fe(3+) ions in association with the binding of an anion, usually bicarbonate.

Lactotransferrin has antimicrobial activity which depends on the extracellular cation concentration. Lactoferroxins A, B and C have opioid antagonist activity. Lactoferroxin A shows preference for mu-receptors, while lactoferroxin B and C have somewhat higher degrees of preference for kappa-receptors than for mu-receptors.

The lactotransferrin transferrin-like domain 1 functions as a serine protease of the peptidase S60 family that cuts arginine rich regions. This function contributes to the antimicrobial activity.

Sequence similarities	Belongs to the transferrin family. Contains 2 transferrin-like domains.
Cellular localization	Secreted.



Images

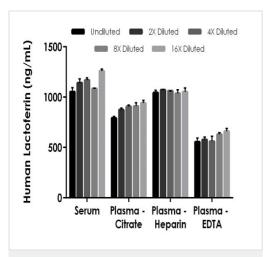
Example of human Lactoferrin standard curve in Sample Diluent NS.

The Lactoferrin standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

Standard Curve Measurements					
Concentration	O.D 4	Mean			
(pg/ml)	1	2	O.D		
0	0.165	0.151	0.158		
156.3	0.225	0.233	0.229		
312.5	0.317	0.319	0.318		
625	0.485	0.479	0.482		
1,250	0.762	0.773	0.767		
2,500	1.361	1.329	1.345		
5,000	2.421	2.384	2.402		
10,000	3.538	3.552	3.545		

Example of human Lactoferrin standard curve in Sample Diluent NS. The Lactoferrin standard curve was prepared. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

Standard curve



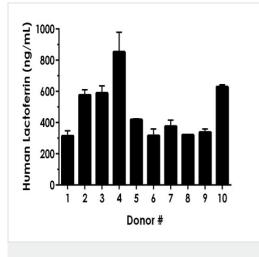
Interpolated concentrations of native Lactoferrin in human serum and plasma samples.

Undiluted 22 Diluted 42 Diluted 

Interpolated concentrations of native Lactoferrin in human milk (defatted), urine, and saliva samples.

The concentrations of Lactoferrin were measured in duplicates, interpolated from the Lactoferrin standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 1:200, plasma (citrate) 1:200, plasma (heparin) 1:200, and plasma (EDTA) 1:200. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Lactoferrin concentration was determined to be 1,142 ng/mL in neat serum, 885.1 ng/mL in neat plasma (citrate), 1,054 ng/mL in neat plasma (heparin), and 581.4 ng/mL in neat plasma (EDTA).

The concentrations of Lactoferrin were measured in duplicates, interpolated from the Lactoferrin standard curves and corrected for sample dilution. Undiluted samples are as follows: milk 1:500,000, urine 1:20, and saliva 1:10,000. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Lactoferrin concentration was determined to be 4,021.6  $\mu$ g/mL in neat milk (defatted), 0.033  $\mu$ g/mL in neat urine, and 39.1  $\mu$ g/mL in neat saliva.



Interpolated dilution factor corrected values are plotted (mean +/-SD, n=2). The mean Lactoferrin concentration was determined to be 473.8 ng/mL with a range of 315.2 – 852.7 ng/mL.

Serum from ten individual healthy human female donors was measured in duplicate.

Dilution Factor	Interpolated value	1:200 Human Serum	1:200 Human Plasma (Citrate)	1:200 Human Plasma (Heparin)	1:200 Human Plasma (EDTA)
Undiluted	pg/mL	5,264	3,960	5,223	2,780
Unaliolea	% Expected value	100	100	100	100
2	pg/mL	2,853	2,187	2,680	1,447
	% Expected value	108	110	103	104
	pg/mL	1,462	1,134	1,327	701.0
4	% Expected value	111	114	102	101
8	pg/mL	677.9	568.8	647.7	394.0
8	% Expected value	103	115	99	113
14	pg/mL	393.5	294.3	329.1	206.3
16	% Expected value	120	119	101	119

Linearity of dilution.

Dilution Factor	Interpolated value	1:500,000 Human Milk	1:20 Human Urine	1:10,000 Human Saliva	100% Cell Culture Media
Undiluted	pg/mL	8,208	1,714	4,323	1,695
unaliutea	% Expected value	100	100	100	100
2	pg/mL	4,147	876.8	1,950	893.7
	% Expected value	101	102	90	105
4	pg/mL	1,964	400.9	984.0	476.8
	% Expected value	96	94	91	113
8	pg/mL	996.9	204.0	463.8	252.7
8	% Expected value	97	95	86	119
16	pg/mL	492.7	97.99	228.9	102.0
10	% Expected value	96	91	85	96

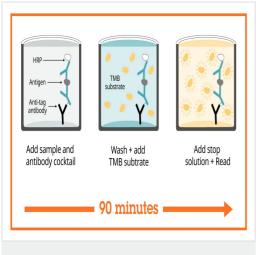
Linearity of dilution.

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

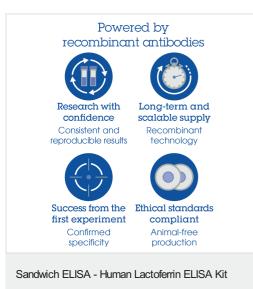
Native Lactoferrin was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Native Lactoferrin was measured in human milk, urine, and saliva samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Native purified Lactoferrin protein was spiked into RPMI 1640 cell culture media containing 10% fetal bovine serum and measured in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.



Sandwich ELISA - Human Lactoferrin ELISA Kit (ab200015)



(ab200015)



Sandwich ELISA - Human Lactoferrin ELISA Kit (ab200015)

SimpleStep ELISA technology allows the formation of the antibodyantigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.

To learn more about the advantages of recombinant antibodies see **<u>here</u>**.

To learn more about the advantages of SimpleStep  $ELISA^{\textcircled{R}}$  kits see **here**.

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