



AssayMax Human Lactoferrin ELISA Kit

Catalog Number EL2011-1

Introduction

Lactoferrin is an 80 kDa iron-binding glycoprotein produced by many exocrine glands with a major constituent in the secondary granules of neutrophilic leukocytes. Serum lactoferrin concentration is much higher during inflammation (1). Lactoferrin is known to be an immune modulator or enhancer due to specific receptors for lactoferrin that are found on many key immune cells such as lymphocytes, monocytes and macrophages, and is known to be directly involved in the up-regulation of natural killer (NK) cell activity (2). Lactoferrin is present in maternal milk, saliva, tears, vaginal secretions, semen, bronchoalveolar lavage fluid, and specific granules of polymorphonuclear leukocytes (PMNs)(3). Lactoferrin is found mainly in the oral cavity where it can come into direct contact with pathogens such as viruses, bacteria, etc. Lactoferrin directly inhibits viruses by binding to viral receptor sites, thus preventing the virus from infecting healthy cells. Lactoferrin has a direct bacteriostatic function to certain bacteria such as *Streptococcus mutans*, *Vibrio cholerae*, *Escherichia coli*, *Actinobacillus actinomycetemcomitans*, and *Legionella pneumophila* (2,3,4). Also, it has a bacteriostatic effect that deprives iron-requiring bacteria of this essential growth nutrient (4). Lactoferrin is also considered an antioxidant that scavenges free iron, helping to prevent uncontrolled iron based free radical reactions, thus protecting certain cells from peroxidation. (2)

Principal of the Assay

The AssayMax Human Lactoferrin ELISA kit is designed for detection of human plasma, serum, urine, saliva, milk and other body fluids. This assay employs a quantitative sandwich enzyme immunoassay technique, which measures lactoferrin in 4 hours. A polyclonal antibody specific for lactoferrin has been pre-coated onto a microplate. Lactoferrin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for lactoferrin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **Human Lactoferrin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human lactoferrin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Lactoferrin Standard:** Human Lactoferrin in a buffered protein base (400 ng, lyophilized).
- **Biotinylated Lactoferrin Antibody (100x):** A 100-fold biotinylated polyclonal antibody against human lactoferrin (80 μ l).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 μ l).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (10x):** A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2-8⁰C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8⁰C. Store reconstituted standard at -20⁰C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 μ l, 20-200 μ l, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:200 into MIX Diluent. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:200 into MIX Diluent. Store serum at -20⁰C or below. Avoid repeated freeze-thaw cycles
- **Saliva:** Rinse your mouth at least twice with cool water. Then chew the sugarless gum provided for 1-2 minutes, swallowing saliva as usual. You may continue to chew the gum during saliva collection but please do not spit it into the collection tube. If, for some reason, you are not able to chew the gum, saliva collection is still possible but may take longer. Dilute samples 1:100000 into MIX Diluent. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- **Urine:** Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:10 into MIX Diluent. Store samples at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20⁰C or below. Avoid repeated freeze-thaw cycles

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **Standard Curve:** Reconstitute the 400 ng of Lactoferrin Standard with 5 ml of MIX Diluent to generate a stock solution of 80 ng/ml. Allow the stock solution to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution can be further dilute with equal volume of MIX Diluent to produce standard solution (40 ng/ml) Prepare triplicate standard points by serially diluting the standard solution (40 ng/ml) 1:4 with MIX Diluent to produce 10, 2.5, 0.625, and 0.156 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at < -20⁰C.

Standard Point	Dilution	[Lactoferrin] (ng/ml)
P1	Standard (40 ng/ml)	40.000
P2	1 part P1 + 3 parts MIX Diluent	10.000
P3	1 part P2 + 3 parts MIX Diluent	2.500
P4	1 part P3 + 3 parts MIX Diluent	0.625
P5	1 part P4 + 3 parts MIX Diluent	0.156
P6	MIX Diluent	0.000

- **Biotinylated Lactoferrin Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer Concentrate 1:10 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30⁰C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Biotinylated Lactoferrin Antibody to each well and incubate for one hour.
- Wash five times with 200 µl of Wash Buffer as above.

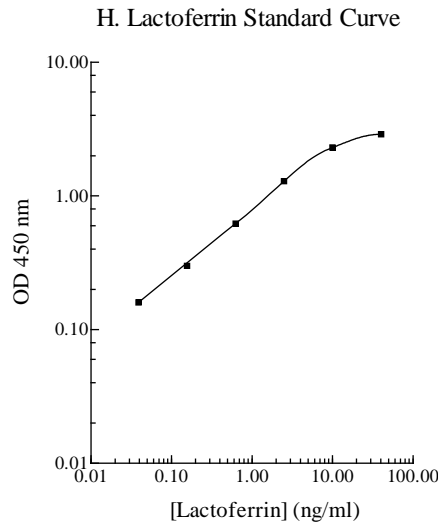
- Add 50 μ l of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 μ l of Wash Buffer as above.
- Add 50 μ l of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μ l of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.
- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or 4-parameter curve fit. Determine the unknown sample concentration from the Standard Curve and multiply the sample value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Precision, Sensitivity and Specificity

- The minimum detectable dose of Lactoferrin is typically 0.1 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.1% and 7.1% respectively.

Cross-Reactivities

Species	% Cross Reactivity
Monkey	< 20 (suggest dilution 1:20 for plasma/serum)
Mouse	< 1
Rat	None
Swine	None
Beagle	< 0.3
Bovine	< 1

- If cell culture supernatants contains 10% FBS, The minimum detectable dose of human lactoferrin will be 1 ng/ml.

Recovery

Standard Added Value	0.5 – 20 ng
Recovery %	85-117 %
Average Recovery %	101 %

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:100	98%	96%
1:200	103%	101%
1:400	106%	100%

Sample Dilution	Average Percentage of Expected Value
	Urine
1:5	100%
1:10	105%
1:20	101%

References

1. Noat, D. *et al.* (2005) *Clinical Medicine & Research*. Vol 3, No. 2: 93-101
2. Brink, W. (October 2000) LE Magazine.
3. Yamauchi, K. *et al* (1993) *Infection and Immunity*. Vol 61, No 2, p.719-728
4. Conneely, O. M. (2001) *J.of the Am. Col. of Nutrition*. Vol. 20, No. 5, 389S-395S

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