Human Lactoferrin ELISA Kit

Cat. No. E88-143
Lot No. E88-143-211019

Components Supplied

- **Lactoferrin Pre-Coated 96-well Strip Plate,** 1 each
- **Lactoferrin Standard**, 200 ng/vial, 2 each
- **Lactoferrin Detection Antibody,** 12 ml
- **10X Dilution Buffer B**, 25 ml
- **HRP Solution A,** 12 ml
- **TMB Substrate,** 12 ml
- **Stop Solution,** 12 ml
- **20X Wash Buffer,** 50 ml
- **Sealing Tape,** 6 sheets

Shelf Life: 6 months from date of sale.

* See pg 5 for important change related to dilution of standard. *

For In vitro laboratory use only. Not for any clinical, therapeutic, or diagnostic use in humans or animals. Not for animal or human consumption.

Introduction

Enzyme linked immunosorbent assay (ELISA) for the detection of Human Lactoferrin in plasma, urine, milk and cerebrospinal fluid (CSF). Kit contains sufficient components to quantitate Human Lactoferrin protein concentration in up to 40 samples, tested in duplicate. **Note:** EDTA or Sodium Citrate plasmas are the preferred blood sample types. Sodium Heparin may interfere with lactoferrin measurement.

Background

Lactoferrin, also known as lactotransferrin, is an iron-binding glycoprotein that belongs to the transferrin family of proteins. This globular protein has a molecular mass of 80 kDa and contains four identical domains. Each pair of domains surrounds an iron (Fe) atom.

First isolated in milk, lactoferrin has been also found in many mucosal secretions, such as tears and saliva. Lactoferrin is also present in secondary granules of polymorphonuclear cells and is also secreted by some acinar cells. Human colostrum has the highest lactoferrin concentration, followed by human milk, and then cow milk. Lactoferrin is generally purified from milk or produced recombinantly.

Lactoferrin is a multifunctional protein that is part of the innate defense against infection and inflammation. Its long known antimicrobial activity is at least partly attributed to the ability of the apoprotein to sequester two atoms of iron, an essential bacterial nutrient. Proteolysis of lactoferrin produces lactoferricin, which has an even enhanced antimicrobial activity. This is attributed to its ability to disrupt the membrane structure, which eventually results in cell lysis.

Other lactoferrin functions include inhibition of binding of bacterial and viral particles into host cells, or with each other. It has also antifungal activity. Lactoferrin receptors have been found on brush-border cells, monocytes, macrophages, and activated lymphocytes. More recently, lactoferrin was found to inhibit dendritic cell-mediated HIV-1 transmission by blocking the interaction of gp120 to DC-SIGN, which is a critical HIV protein that never changes regardless of strain {Groot F. et al. (2005) *J. Virol.*, 79(5), 3009-15}. These broad ranges of functions clearly reflect the biological importance of lactoferrin.

In humans, the lactoferrin gene is located on chromosome 3; location: 3q21-q23.

Principle of the Assay

This kit is based on a sandwich ELISA. Human Lactoferrin present in the test sample is captured by anti-human lactoferrin antibody that has been pre-adsorbed on the surface of microtiter wells. After sample binding unbound proteins and molecules are washed off, and a biotinylated detection antibody is added to the wells to bind to the captured lactoferrin. A strepavidin-conjugated horseradish peroxidase (SA-HRP) is then added to catalyze a colorimetric reaction with the chromogenic substrate TMB (3,3',5,5'-tetramethylbenzidine). The colorimetric reaction produces a blue product, which turns yellow when the reaction is terminated by addition of dilute sulfuric acid. The absorbance of the yellow product at 450 nm is proportional to the amount of lactoferrin analyte present in the sample and a four-parameter standard curve can be generated. The lactoferrin concentrations in the test samples can then be quantified by interpolating their absorbance from the standard curve generated in parallel with the samples. After factoring sample dilutions the lactoferrin concentrations in the original sample can finally be calculated.

Procedure Overview

1. Add 100 µl of standard or sample to well.
   Note: Run each standard or sample in duplicate.

2. Cover plate and incubate at room temperature (20-25°C) for 1 hour.

3. Wash plate FOUR times.

4. Add 100 µl of Lactoferrin Detection Antibody to each well.

5. Cover plate and incubate at room temperature for 1 hour.

6. Wash plate FOUR times.

7. Add 100 µl of HRP Solution A to each well.

8. Cover plate and incubate at room temperature for 30 minutes.

9. Wash plate FOUR times.

10. Add 100 µl of TMB Substrate Solution to each well.

11. Develop the plate in the dark at room temperature for 30 minutes.

12. Stop reaction by adding 100 µl of Stop Solution to each well.

13. Measure absorbance on a plate reader at 450 nm.

Additional Materials Required

- Ultrapure water
- Precision pipettors, with disposable plastic tips
- Polypropylene or polyethylene tubes to prepare standard and samples − do not use polystyrene, polycarbonate or glass tubes
- A container to prepare 1X Dilution Buffer B
- A container to prepare 1X Wash Buffer
- A wash bottle or an automated 96-well plate washer
- Disposable reagent reservoirs
- A standard microtiter plate reader for measuring absorbance at 450 nm

Precautions

- Store all reagents at 2-8ºC. Do not freeze reagents.
- All reagents must be at room temperature (20-25°C) before use.
- Vigorous plate washing is essential.
- Use new disposable pipette tips for each transfer to avoid cross-contamination.
- Use a new adhesive plate cover for each incubation step.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Avoid microbial contamination of reagents and equipment. Automated plate washers can easily become contaminated thereby causing assay variability.
- Take care not to contaminate the TMB Solution. Do not expose TMB Substrate solution to glass, foil, or metal. If the solution is blue before use, DO NOT USE IT.
- Individual components may contain preservatives. Wear gloves while performing the assay. Please follow proper disposal procedures.

Reagent, Standard, and Sample Handling and Preparation

1X Dilution Buffer B Preparation

- Prepare 1X Dilution Buffer B by diluting 25 ml of 10X Dilution Buffer B into 225 ml of ultra pure water. Mix well. Store reconstituted 1X Dilution Buffer B at 2-8ºC for up to six (6) months. Do not use 1X Dilution Buffer B if it becomes visibly contaminated during storage.

Standard Preparation

- Standard should be treated as a biological material and universal precautions should be followed.

1. Reconstitute 200 ng standard vial with 1 ml of 1X Dilution Buffer B to achieve a final concentration of 200 ng/ml. Mix well.
2. Label eight (8) tubes, one for each standard curve point: 100 ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.13 ng/ml, 1.56 ng/ml, and 0 ng/ml.
3. Prepare top standard of 100 ng/ml by diluting 500 µl of 200 ng/ml standard with 500 µl of 1X Dilution Buffer B. Mix well.
4. Pipette 500 µl of 1X Dilution Buffer B into remaining tubes.
5. Serially dilute the 100 ng/ml standard 1:1 with 1X Dilution Buffer B. Perform dilution by mixing 500 µl of the previous standard with 500 µl of 1X Dilution Buffer B. Continue until reach standard value of 1.56 ng/ml.
6. Use 1X Dilution Buffer B only as the zero standard value.

Sample Handling

- Plasma, urine, milk and cerebrospinal fluid may be tested in this ELISA. EDTA or Sodium Citrate plasmas are the preferred blood sample types. Sodium Heparin may interfere with lactoferrin measurement.
- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing of infectious agents.
- 100 µl of sample or standard is required per well.
- Samples must be assayed in duplicate each time the assay is performed.

- Store samples to be assayed within 24 hours at 2-8ºC. For long-term storage, aliquot and freeze samples at -70°C. Avoid repeated freeze-thaw cycles when storing samples.
- If particulate is present in samples, centrifuge prior to analysis.
- If samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note on the Plate Template and interpret results with caution

Sample Preparation

- **Plasma samples must be diluted with 1X Dilution Buffer B prior to testing**.
- EDTA plasma samples require a 1:20 dilution with 1X Dilution Buffer B. A 1:20 dilution could be prepared by adding 13 µl of sample to 247 µl of 1X Dilution Buffer B in a separate tube and mixing well.
- Sodium Heparin and Sodium Citrate Plasma samples require a 1:5 dilution with 1X Dilution Buffer B. A 1:5 dilution could be prepared by adding 50 µl of sample to 200 µl of 1X Dilution Buffer B in a separate tube and mixing well.
- Cerebrospinal Fluid (CSF) may be assayed undiluted. If concentration of Lactoferrin is suspected to be higher than 100 ng/ml, dilution of sample should be made with Dilution Buffer B.
- If it is suspected that the concentration of the unknown sample (for example, a milk sample) exceeds the highest point of the standard curve, prepare one or more dilutions of the sample in 1X Dilution Buffer B until the desired concentration is obtained. For example, if a final dilution of 1:10,000 is desired, an initial 1:100 dilution could be prepared by adding 10 µl of sample to 990 µl of 1X Dilution Buffer B in a separate tube and mixing well. The final 1:10,000 dilution could be prepared by adding 10 µl of the 1:100 sample to 990 µl 1X Dilution Buffer B in a separate tube and mixing well.

1X Wash Buffer Preparation

- Prepare 1X Wash Buffer by diluting 20X Wash Buffer in ultra pure water. For example, if preparing 1 L of 1X Wash Buffer, dilute 50 ml of 20X Wash Buffer into 950 ml of ultrapure water. Mix well. Store reconstituted 1X Wash Buffer at 2-8ºC for up to six (6) months. Do not use 1X Wash Buffer if it becomes visibly contaminated during storage.

Assay Procedure

Sample Incubation

- Determine the number of strips required. Leave these strips in the plate frame. Place unused strips in the foil pouch with desiccant and seal tightly. Store unused strips at 2-8°C. After completing assay, keep the plate frame for additional assays.
- Use a Plate Template to record the locations of the standards and unknown samples within the wells.

1. Add 100 µl of appropriately diluted standards or samples to each well. Run each standard, sample, or blank in duplicate.

   Note: Plasma and milk samples must be diluted prior to testing (see Sample and Reagent Preparation-Sample Dilution section).
2. Carefully cover wells with a new adhesive plate cover. Incubate for one (1) hour at room temperature, 20-25°C.
3. Carefully remove adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.

Plate Washing

- Using a manual or automated plate washer

1. Rinse the tips of the plate washer by dispensing the 1X Wash Buffer into the wash trough and aspirating the solution. Repeat this step five times. For automated plate washers, program the rinse step accordingly.

   Note: This initial rinse step is necessary especially if the plate washer has been idle for several days or longer. Automated plate washers are susceptible to microbial growth in the fluid lines and cavities.
2. Aspirate the solutions from the wells. Fill the wells to about 90% full with 1X Wash Buffer and then aspirate the wash solution. Repeat this wash step three more times. For automated plate washers, program 4 washes at 300 µl per wash, according to the manufacturer's instructions.

- Using a squirt bottle

1. Gently squeeze the long sides of plate frame before washing to ensure all strips remain securely in the frame.
2. Empty the plate contents by quickly dumping the contents of the wells into the sink using a quick, flipping motion.

3. Use a squirt wash bottle to fill each well completely with 1X Wash Buffer, and then empty the plate contents. Repeat procedure three more times for a total of FOUR washes. Blot the plate onto paper towels or other absorbent material.

Detection Antibody Incubation

- Only remove the required amount of Detection Antibody reagent for the number of strips being used.

1. Add 100 µl of appropriate Detection Antibody to each well containing standard, sample or blank. Mix well by gently tapping the plate several times.
2. Carefully attach a new adhesive plate cover. Incubate plate for one (1) hour at room temperature, 20-25°C.
3. Carefully remove the adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.

HRP Solution A Incubation

- Only remove the required amount of HRP Solution A for the number of strips being used.

1. Add 100 µl of HRP Solution A to each well containing sample or blank.
2. Carefully attach a new adhesive plate cover. Incubate plate for 30 minutes at room temperature, 20-25°C.
3. Carefully remove the adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.

TMB Substrate Incubation and Reaction Stop

- Only remove the required amount of TMB Substrate Solution and Stop Solution for the number of strips being used.
- Do NOT use a glass pipette to measure the TMB Substrate Solution. Do NOT cover the plate with aluminum foil or metalized mylar. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate Solution. If the solution is blue before use, DO NOT USE IT!

1. Add 100 µl of TMB Substrate Solution into each well.

2. Allow the enzymatic color reaction to develop at room temperature (20-25ºC) in the dark for 30 minutes. Do NOT cover plate with a plate sealer. The substrate reaction yields a blue solution.
3. After 30 minutes, stop the reaction by adding 100 µl of Stop Solution to each well. Tap plate gently to mix. The solution in the wells should change from blue to yellow.

Absorbance Measurement

Note: Evaluate the plate within 30 minutes of stopping the reaction.

1. Wipe underside of wells with a lint-free tissue.
2. Measure the absorbance on an ELISA plate reader set at 450 nm.

Calculation of Results

- Duplicate absorbance values should be within 10% of each other. Care should be taken when interpreting data with differences in absorbance values greater than 10%.

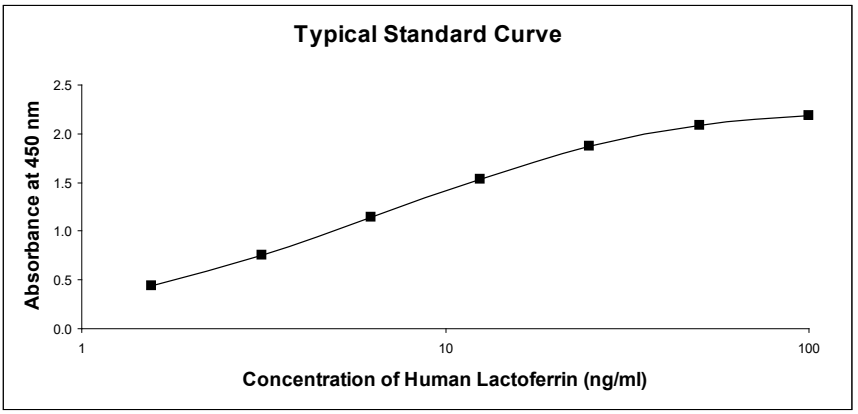
1. Prepare a standard curve to determine the amount of lactoferrin in an unknown sample. Plot the average absorbance obtained for each standard concentration on the vertical (Y) axis versus the corresponding lactoferrin concentration on the horizontal (X) axis using curve-fitting software.
2. Calculate the Lactoferrin concentration in unknown samples using the prepared standard curve. Determine the amount of lactoferrrin in each unknown sample by noting the lactoferrin concentration (X axis) that correlates with the absorbance value (Y axis) obtained for the unknown sample.
3. If the sample was diluted, multiply the observed lactoferrin concentration by the dilution factor to determine the concentration of Lactoferrin in the original, undiluted sample.

   Note: Most plate readers come with appropriate templates and curve-fitting software. The standard curve of this assay can be fitted into a 4-parameter curve fitting equation that can be programmed to calculate and display a table (or tables) consisting of the raw absorbance readings, net absorbance readings, the analyte concentration in the assay solution, dilution factors, and analyte concentration in the original unknown sample.

Performance Characteristics

Typical Standard Curve

- This typical standard curve was generated using the Human Lactoferrin ELISA Kit Protocol. This standard curve is for demonstration only. A standard curve must be generated for each assay.



Assay Range: 1.56 – 100 ng/ml

- Suggested standard curve points are 100 ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.13 ng/ml, 1.56 ng/ml, and 0 ng/ml.

Specificity

- This ELISA is specific for Human Lactoferrin. It does not cross-react with Transferrin, Bilirubin, Hemoglobin, Albumin, Casein, or Ferritin. Cross-reactivity with bovine lactoferrin is less than 1%.

Representative Data

- Plasma, urine, and milk samples were collected from apparently healthy individuals and evaluated in this assay. CSF samples, collected from unhealthy individuals, are clinically normal. The levels of Human Lactoferrin detected are as follows:

Human Plasma (n=6):

Note: EDTA or Sodium Citrate plasmas are the preferred blood sample types. Sodium Heparin may interfere with lactoferrin measurement.

	Human Lactoferrin Concentration (ng/ml)					
	Donor 1	**Donor 2**	**Donor 3**	**Donor 4**	**Donor 5**	**Donor 6**
EDTA Plasma	90.9	86.3	119.4	140.2	50.3	134.3
Sodium Citrate Plasma	63.9	78.2	72.9	88.6	40.0	156.4
Sodium Heparin Plasma	31.9	26.5	30.0	29.9	16.8	43.4
CSF*	3.27	188.68	8.99	2.6	3.9	-

Human Urine (n=1): 2.4 ng/ml

Human Milk (n=1): 3.7 mg/ml

*** Donors of CSF were not the same donors of the plasma samples.**

Warranty

Products are warranted by Bethyl Laboratories, Inc. to meet stated product specifications and to conform to label descriptions when used, handled and stored according to instructions. Unless otherwise stated, this warranty is limited to six months from date of sale. Bethyl Laboratories sole liability for the product is limited to replacement of the product or refund of the purchase price. Bethyl Laboratories products are supplied for research applications. They are not intended for medicinal, diagnostic or therapeutic use. The products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from Bethyl Laboratories, Inc.

Rev 201018

Plate Templates

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												