*Oxis*Research™

LumiQuick Diagnostics, Inc.

BIOXYTECH® Lacto*ƒ* EIA™

Assay for Human Lactoferrin
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Catalog Number A21015 (AOXRE Catalog Number 21015)

INTRODUCTION

The Analyte

Human lactoferrin (LTF) is an 80 kDa glycoprotein which was first isolated from human milk. LTF is found in most body fluids and secretions, *e.g.*, in the nose, genital tract, and tears.[1] In the blood, LTF is secreted by neutrophils and its plasma concentration is positively related to the total pool of neutrophils and to the rate of neutrophil turnover.[2] Because of its ability to strongly bind iron, LTF is considered to be bactericidal.[3] In a number of cases of inflammation, LTF is released into the extracellular medium from secondary granules of neutrophilic leukocytes.[4,5] Its extracellular concentration can therefore be used as an index of neutrophil activation, especially in blood samples containing anti-myeloperoxidase antibodies.

Principle of the Procedure

The BIOXYTECH® Lacto*ƒ*-EIA™ method is an enzyme-linked immunosorbent assay (ELISA). Lactoferrin is captured by a monoclonal antibody (MAb) that is coated on wells of a sectional microplate. A second LTF-MAb labeled with biotin is added to the well and binds with the captured LTF forming a "sandwich." A solution of streptavidin-peroxidase is then added. Streptavidin has a high affinity for biotin and once bound, its horseradish peroxidase (HRP) label is available for color development by addition of the substrate, *o*-phenylenediamine (OPD). This color development at 450 nm is proportional to the quantity of LTF in the sample.

REAGENTS

Materials Provided (for 96 tests)

• Sample Diluting Buffer (1)	Phosphate buffer containing NaCl, bovine serum albumin (BSA), Tween-20, and 0.01% thimerosal. This solution is used to dilute biological samples. 3 x 25 mL
• LTF Standard (2)	Approximately 300 ng of purified LTF with BSA in lyophilized form. 1 vial
• Wash Buffer (3)	Tris-HCl buffer containing NaCl, Tween-20, and 0.01% thimerosal. 100 mL 20x
• Anti-LTF Solution (4)	Concentrated solution of monoclonal antibody to LTF in phosphate buffer containing NaCl, BSA, glycerol, and 0.01% thimerosal. 75 µL
• Streptavidin-HRP (5)	Concentrated solution of streptavidin-coupled horseradish peroxidase in Tris-HCl buffer containing BSA and 0.01% thimerosal. 75 µL
• Diluting Buffer (6)	Phosphate buffer containing NaCl, BSA, Tween-20, and 0.01% thimerosal. For diluting solutions (4) and (5). 3 x 8 mL
• OPD Diluting Buffer (7)	Citrate buffer containing H_2O_2 and 0.01% thimerosal. 20 mL
• Stop Solution (8)	1 M H_2SO_4. 20 mL
• OPD (9)	Four tablets.
• Microplates	One resealable packet containing 6 x 16 well strips plus frame.
• Plate Sealers	4 self-adhesive sheets

Materials Required But Not Provided

- Deionized water
- Test tubes and beakers
- Adjustable pipettes (50-1000 µL)
- Incubator (37±1°C)
- Microplate reader for absorbance measurements at 450 nm

Warnings and Precautions

- For *in vitro* use only.
- In case of accidental exposure of skin, eyes, or mucous membranes to Stop Solution (8), wash the exposed area thoroughly with water for 15 minutes.
- The final concentration of thimerosal is 0.01%.
- Human LTF was purified from human milk and should be handled with standard universal precautions**.** Standard (2) solution should be manipulated with the same precautions as that usually required for other products of human origin.

PROCEDURE

Reagent Preparation

Solutions (1), (3), (6), (7), and (8) should be placed at ambient temperature for 30 minutes before use. Buffers (1) and (6) should be used within 5 days after opening of bottles.

LTF Standard (2):

Prepare a solution of LTF standard (100 ng/mL) by adding 3 mL of buffer (1) to the lyophilized protein. The solution may be stored at 2-8°C for 3 days. For longer storage, prepare aliquots immediately and store them at -20°C.

Wash Buffer 20X (3):

This solution should be diluted 20 times prior to use. Example dilutions, based on the required amount, are described in table 1. The diluted Wash Buffer is stable for 5 days at ambient temperature.

Table 1: Dilution Volumes For Wash Buffer

Number of 16 Well Strips	Required Volume of Wash Buffer	Volume of Concentrated Wash Buffer 20X (3)	Volume of Deionized Water
2	250 mL	12.5 mL	237.5 mL
4	500 mL	25 mL	475 mL
6	1000 mL	50 mL	950 mL

Anti-LTF Solution (4) and Streptavidin HRP (5):

These solutions must be freshly diluted 250 times in Diluting Buffer (6) prior to use. Example dilutions, based on the required amount, are described in table 2.

Table 2: Dilution Volumes For Solution (4) And (5)

Number of 16 Well Strips	Volume of Diluting Buffer (6)	Volume of Solution (4) or (5)
2	4 mL	16 µL
4	8 mL	32 µL
6	12 mL	48 µL

OPD Solution:

Five minutes before use, dissolve the required number of OPD tablets in OPD Diluting Buffer (7). Examples of some dilutions are described in table 3.

Table 3: Dilution Volumes For OPD Tablets And Buffer

Number of 16 Well Strips	Volume of OPD Diluting Buffer (7)	Number of OPD Tablets (9)
2	5 mL	1
4	10 mL	2
6	15 mL	3

Assay

Microtiter Plate Preparation

- Determine the number of wells required from the number of samples to assay plus the eight levels of standard times the number of replicates you intend to run.
- Use only the number of 16 well strips required. Remove unneeded strips from the frame and place in the resealable foil packet. Store at 2-8°C for future use.
- After assay, keep the frame for future use.

Standard Preparation

- Perform a serial dilution of the 100 ng/mL Standard to the following concentrations: 50, 25, 12.5, 6.2, 3.1 and 1.6 ng/mL.
- Pipet 100µL Standard or sample to the appropriate well.
- Cover the wells with a Plate Sealer and incubate at 37°C for one hour. (± 5 minutes).

Anti-LTF incubation

- Prepare the required volume of diluted Anti-LTF Solution (4) (table 2).
- Empty the microplate.
- Wash wells 5 times with diluted Wash Buffer (3).
- Blot residual liquid on absorbent paper.
- Pipet 100 µL of diluted anti-LTF Solution into each well.
- Cover the wells with Plate Sealer and incubate at 37°C for 1 hour. (± 5 minutes)

Streptavidin-HRP incubation

- Prepare the required volume of Streptavidin-HRP solution (5) (table 2).
- Empty the microplate.
- Wash wells 5 times with diluted Wash Buffer (3).
- Blot residual liquid on absorbent paper.
- Pipet 100 µL of diluted Streptavidin-HRP into each well.
- Cover the wells with Plate Sealer and incubate at 37°C for 15 minutes.

Colorimetric measurement

- Prepare the required volume of OPD solution (table 3).
- Empty the microplate.
- Wash wells 5 times with diluted Wash Buffer (3).
- Blot residual liquid on absorbent paper.
- Pipet 100 µL of prepared OPD solution into each well.
- Cover the wells with Plate Sealer and incubate at 37°C until the absorbance of the 100 ng/mL Standard reaches about 1-1.5 (approximately 5 to 10 minutes).
- Add 50 µL of Stop Solution (8) to each well.
- Read the absorbance at 450 nm.

Results and Calculations

The standard curve is obtained by fitting the standard absorbance at 450 nm to the concentration of LTF by 4-parameter logistic curve fit. See figure 1.

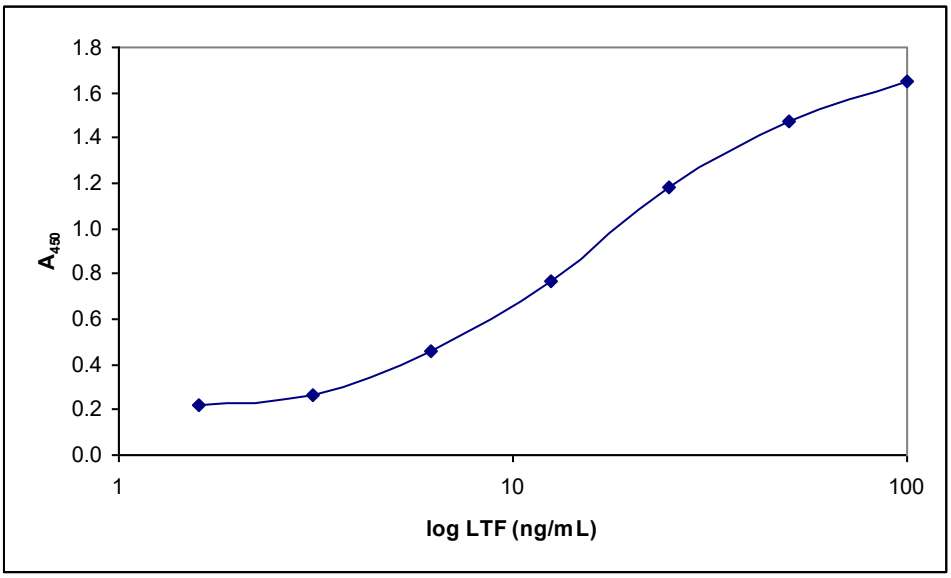

Figure 1: A typical standard curve obtained using the 4-parameter logistic curve fit.

NOTES

Examples Of Sample Preparations

Samples should be diluted, if necessary, using Sample Diluting Buffer (1). 100 µL of sample is used for each determination.

Plasma:

1. Draw blood in a tube containing EDTA as anticoagulant* and keep at 4°C.
2. Centrifuge whole blood within 6 hours at 3,000 x *g* for 10 minutes at 4°C.
3. Remove the plasma supernatant.**
4. Dilute plasma 40-fold (v/v) in Sample Diluting Buffer (1).***
5. Use 100 µL of diluted sample for the assay.

* Heparin may interfere with LTF measurements.
** Plasma samples can be stored at 4°C for 24 hours. For longer storage, samples should be stored at -20°C. Avoid repeated freezing/thawing.
*** Further dilution is needed with samples containing high concentrations of LTF.

Urine:

1. Collect urine in clean flasks.
2. Centrifuge at 1,000 x *g* for 10 minutes at 4°C.
3. Remove the supernatant*.
4. Use 100 µL of the supernatant for each determination.**

* The supernatant can be stored at 4°C for 3 days.
** At LTF concentrations above 100 ng/mL, dilute the sample with Sample Diluting Buffer(1).

Broncho Alveolar Lavage (BAL)

The assay of LTF can be performed without prior dilution of BAL, unless the measured concentration of LTF in such samples is higher than 100 ng/mL. For higher concentrations of LTF, BAL samples should be diluted with Sample Diluting Buffer (1).

Cerebrospinal Fluid (CSF)

The assay of LTF can be performed without prior dilution of CSF, unless the measured concentration of LTF in such samples is higher than 100 ng/mL. For higher concentrations of LTF, CSF samples should be diluted with Sample Diluting Buffer (1).

Supernatants after centrifugation of cell cultures

The concentration of lactoferrin in cell culture medium can be measured without prior dilution of the medium, unless the measured concentration of LTF is higher than 100 ng/mL. For higher concentrations of LTF, the medium should be diluted with sample diluting buffer (1).

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