

COW LACTOFERRIN ELISA

Life Diagnostics, Inc., Catalog Number: LF-11

INTRODUCTION

Lactoferrin is a non-heme iron binding glycoprotein found in milk, other secretory fluids and blood. As a component of host defense, it has antimicrobial and antiinflammatory activity. It is a biomarker of mastitis in cattle.^{1,2} Lactoferrin levels range from less than 0.05 mg/ml in normal milk to more than 8 mg/ml in milk from animals with mastitis.

PRINCIPLE OF THE ASSAY

The assay uses affinity purified cow lactoferrin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated cow lactoferrin antibodies for detection. Standards and diluted samples are incubated in the microtiter wells for 45 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. This results in lactoferrin molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB is added and incubated for 20 minutes. If lactoferrin is present a blue color develops. Color development is stopped by the addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of lactoferrin is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Lactoferrin antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- Lactoferrin stock (lyophilized)
- 20x Wash solution; TBS50-20, 50 ml
- 10x Diluent; YD25-10, 25 ml
- TMB, TMB11-1, 11 ml
- Stop solution, SS11-1, 11 ml

Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

STORAGE

The unused kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one volume of the 10x stock with nine volumes of distilled or deionized water.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use, dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The lactoferrin stock is provided lyophilized. Add the volume of distilled or deionized water indicated on the vial label and mix gently until dissolved (*the reconstituted stock should be aliquoted and frozen at or below -20°C if additional use is intended*).
2. Label 7 polypropylene or glass tubes as 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 ng/ml
3. In the tube labeled 100 ng/ml, prepare the 100 ng/ml standard by diluting the reconstituted stock with 1x diluent as detailed on the stock label.
4. Dispense 250 µl of 1x diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.13, and 1.56 ng/ml.
5. Prepare a 50 ng/ml standard by diluting and mixing 250 µl of the 100 ng/ml standard with the 250 µl of diluent in the tube labeled 50 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

Milk: For optimum results, milk should be processed to skim milk or whey. Skim milk can be obtained by centrifugation of milk at $\geq 3000g$ for ~ 15 min at 4°C. Whey is prepared by adjustment of skim milk to pH 4.5 with glacial acetic acid followed by centrifugation and readjustment of the supernatant pH to 6.5 – 7.5 using 5 M NaOH. To obtain values within the range of the standard curve we suggest that samples be diluted 10,000-fold using the following procedure for each sample to be tested.

1. Dispense 495 µl of 1x diluent into two tubes.
2. Pipette and mix 5.0 µl of sample into the first tube and mix. This provides a 100-fold dilution.
3. Mix 5.0 µl of the 100-fold diluted sample with the 495 µl of diluent in the second tube. This provides a 10,000-fold dilution.

Serum: Lactoferrin levels in normal serum are ~100 ng/ml. Samples must be diluted 20-fold or more prior to assay to avoid matrix effects.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 µl of standards and samples into the wells (we recommend that standards and samples be run in duplicate).

3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µl/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 µl of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 µl of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
12. After 20-minutes, stop the reaction by adding 100 µl of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

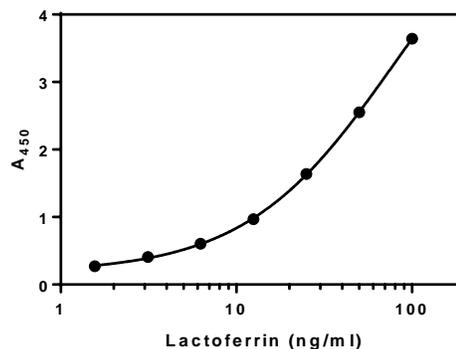
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus \log_{10} of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = \log_{10} concentration) and determine the concentration of the samples from the standard curve (remember to derive the concentration from the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma sample.
4. If the A_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with absorbance at 450 nm on the Y-axis against lactoferrin concentrations on the X-axis is shown below. This curve is for illustration only.

Lactoferrin (ng/ml)	A_{450}
100	3.641
50	2.553
25	1.638
12.5	0.967
6.25	0.606
3.13	0.408
1.56	0.270



REFERENCES

1. Kawai K, Hagiwara S, Anri A, Nagahata H. Lactoferrin concentration in milk of cow clinical mastitis. *Vet Res Commun.* 23(7):391-398 (1999)
2. Harmon RJ et'al. Changes in lactoferrin, immunoglobulin G, cow serum albumin, and a-lactalbumin during acute experimental and natural coliform mastitis in cows. *Infection and Immunity.* 13:533-542 (1976)

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For technical assistance please email us at
techsupport@lifediagnosics.com