KAMIYA BIOMEDICAL COMPANY

Bovine Lactoferrin ELISA

For the quantitative determination of lactoferrin in bovine serum or milk

Cat. No. KT-668

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The ***K-ASSAY®*** Bovine Lactoferrin ELISA is an enzyme immunoassay for the quantitative determination of lactoferrin in bovine serum or milk. For research use only.

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 ant-inflammatory activity. It is a biomarker of mastitis in cattle. 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
The assay uses affinity purified bovine lactoferrin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated bovine lactoferrin antibodies for detection. Calibrators 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 calibration curve.

COMPONENTS
- Lactoferrin antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 mL
- Lactoferrin calibator (lyophilized)
- 20X Wash solution, 50 mL
- 10X Diluent, 25 mL
- TMB, 11 mL
- Stop Solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper or paper towels
- Curve fitting software
- Polypropylene or glass tubes
- Plate reader capable of measuring absorbance at 450 nm
- Plate incubator/shaker
- Plate washer

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 de-ionized 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 de-ionized water.

CALIBRATOR PREPARATION

1. The lactoferrin calibrator is provided lyophilized. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved ***(the reconstituted calibrator 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 calibrator by diluting 105.0 µL of the reconstituted stock with 395.0 µL of 1X diluent.
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 calibrator by diluting and mixing 250 µL of the 100 ng/mL calibrator with 250 µL of diluent in the tube labeled 50 ng/mL.
6. Similarly prepare the remaining calibrators 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 ≥3,000 g 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 calibration 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 the 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 approximately 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 calibrators and samples into the wells (we recommend that calibrators 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 calibration curve by plotting absorbance values of the calibrators versus $\log_{10}$ of the concentration.
2. Fit the calibration curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the calibration 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 milk sample.
4. If the A_{450} values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

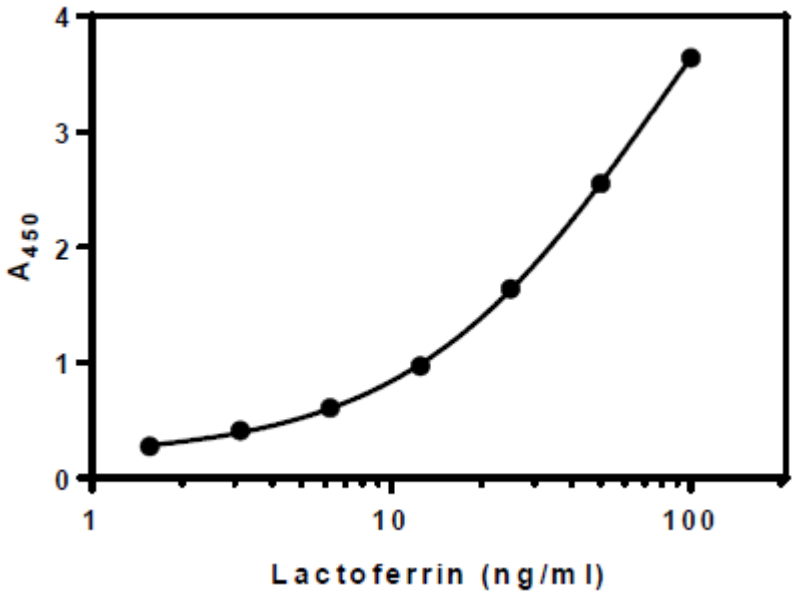
GENERAL INSTRUCTIONS

1. 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.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. All reagents should be allowed to reach room temperature before use.
4. Laboratory temperature will influence absorbance readings. This ELISA kit is calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

TYPICAL CALIBRATION CURVE

A typical calibration 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



STORAGE

The unused kit should be stored at 4℃ and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date.

FOR RESEARCH USE ONLY

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