

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 antiinflammatory activity. It is an excellent biomarker of mastitis in cattle. Lactoferrin levels may 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 bovine lactoferrin test kit is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-bovine lactoferrin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-bovine lactoferrin antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed, and 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-labeled antibodies, and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of lactoferrin is proportional to the optical density of the test sample.

COMPONENTS

- Anti-Bovine Lactoferrin Coated Microtiter Plate (provided as 12 detachable strips of 8 wells)
- Enzyme Conjugate Reagent, 11 mL
- Reference calibrator (lyophilized)
- 20X Wash Buffer, 50 mL
- 10X Diluent, 25 mL
- TMB Reagent (One-step), 11 mL
- Stop Solution (1N HCl), 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes and tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper or paper towels
- Graph paper (PC graphing software is recommended)
- Polypropylene or glass tubes
- Plate reader with an optical density range of 0-4 at 450 nm.
- Micro-Plate incubator/shaker mixing speed of ~150 rpm
- 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 (1) volume of the 10x stock with nine (9) 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 bovine lactoferrin calibrator is provided as a lyophilized stock. 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 -20°C after reconstitution if future 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 the reconstituted stock with 1X diluent as detailed on the calibrator label.
4. Dispense 250 μ L 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 μ L of the 100 ng/mL calibrator with 250 μ L of diluent in the tube labeled 50 ng/mL.
6. Similarly prepare the 25, 12.5, 6.25, 3.13, and 1.56 ng/mL calibrators by 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 $\geq 3,000$ 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. In order 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 μ L of 1X diluent into two tubes.
2. Pipette and mix 5.0 μ L of the sample into the first tube and mix. This provides a 100 fold diluted sample.
3. Mix 5.0 μ L of the 100 fold diluted sample with the 495 μ L of diluent in the second tube. This provides a 10,000 fold dilution of the sample.

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

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μ L of calibrators and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
4. Wash and empty the microtiter wells 5 times with 1x wash solution. This should preferably be performed using a plate washer (400 μ L/well). If a plate washer is not available, use a squirt bottle. The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 μ L of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
8. Wash as detailed in steps 4 to 5 above.
9. Dispense 100 μ L of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
11. Stop the reaction by adding 100 μ L of Stop Solution to each well.
12. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
13. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes. ***In the event that the calibrators or samples exceed the absorbance range of your plate reader, optical density may be determined at 405 nm.***

CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis. ***Please note that the 1.56 - 50 ng/mL data points are optimum for curve fitting. On the discretion of the user, the 100 ng/mL calibrator can be omitted.***
3. Using the mean absorbance value for each sample, determine the corresponding concentration of lactoferrin in ng/mL from the calibration curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of lactoferrin in the

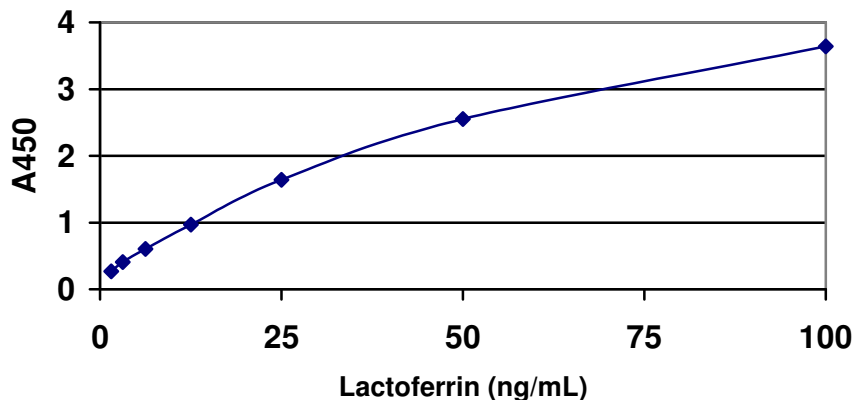
sample.

5. PC graphing software should preferably be used for the above steps. We find that a second order polynomial model gives a good fit of the calibrator data.
6. If the OD₄₅₀ values of the sample fall outside the calibration curve when tested at a 10,000 fold dilution, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density readings at 450 nm on the Y axis against lactoferrin concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and calibration curve in each experiment.

Lactoferrin (ng/mL)	Absorbance (450 nm)
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 kit should be stored at 4 °C, and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable until the expiration date provided that the components are stored as described above.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions detailed above.
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 (18-25 °C) before use.

FOR RESEARCH USE ONLY

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