Bovine Lactoferrin ELISA Kit

bLF ELISA

Product #: E6001-96T

Manufacturer Information

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1. Introduction

Lactoferrin (LF), also known as lactotransferrin (LTF), is a multifunctional protein of the transferrin family. Apart from its main biological function, namely binding and transport of iron ions, lactoferrin also has antibacterial, antiviral, antiparasitic, catalytic, anti-cancer, and anti-allergic functions and properties.

2. Principle of the Test

This ELISA Kit is based on indirect competitive ELISA to detect bovine lactoferrin (bLF) in milk and milk products.

3. Scope of Application

This kit is applicable for determination of bovine lactoferrin in milk / milk powder

4. Kit components

1) Microtiter plate, 96wells, 1 plate
2) bLF standards, 1mL/vial, 0, 0.5, 1.5, 4.5, 13.5, 40.5 μg/mL
3) Enzyme conjugate, 12mL, with brown cap
4) bLF Antibody, 7mL, with green cap
5) Rinsing Buffer, 10mL, with transparent cap
6) TMB Substrate, 12mL, with brown cap
7) Stop solution (not provided)
8) 20x Wash buffer, 50mL, with transparent cap
9) 2x Sample buffer, 50mL, with transparent cap

5. Required Reagents and Instrument (not provided)

1) Incubator (adjustable temperature is 25 ◦C)
2) ELISA reader, with 450/630nm
3) Vortex mixer
4) Analytical balance: accuracy 0.0001g
5) Centrifuge tube, 2mL, 15mL, 50mL
6) Micropipette, 5-50μL, 20-200μL, 100-1000μL
7) Multi-channel pipette, 250μL
8) Ultrapure water

6. Buffer preparation

6.1 Buffer 1: wash buffer

Dilute 20x wash buffer with ultrapure water, in the volume ratio of 1:19, fox example, 10mL 20x wash buffer + 190mL ultrapure water, mix thoroughly.

This diluted wash buffer can be stored at (2-8◦C/36-46 ◦F) for 1 month.

6.2 Buffer 2: sample buffer

Dilute 2x sample buffer with deionized water, in the volume ratio of 1:1, fox sample, 10mL 2x sample buffer + 10mL ultrapure water, mix thoroughly.

This diluted sample buffer can be stored at (2-8◦C/36-46 ◦F) for 1 month.

7. Sample preparation

7.1 Precautions before prepare samples:

1) Use disposable tips during the test. Change new tip for different sample / reagent.
2) Make sure all lab wares are clean and ready to use.
3) Prepared sample shall be analyzed immediately after dilution.

7.2 Milk powder

Take 1±0.0010g milk powder into 15mL centrifuge tube, dissolve with 10mL deionized water, vortex for 15s to dissolve thoroughly (the dilution factor is 10).
Then further dilute the prepared sample solution with ultrapure water so that the final concentration of lactoferrin is in the guidance range*. During each dilution, vortex for 15s.

7.3 Raw milk

Take 100μl raw milk into 2ml centrifuge tube, dilute with 1.1ml sample buffer, vortex for 15s (the dilution factor is 12).
*Please refer to the technical supplementary instructions for lactoferrrin test kit for the guidance range.

8. Assay procedures

1) Return the ELISA kit and all reagents to room temperature (20-25 ◦C /68-77 ◦F). For example, keep these reagent and kits at room temperature for at least 30min.
2) Take needed microwells and zip the rest microwells in the zip-bag and return to 2-8◦C/36-46 ◦F.
3) Layout the plate and record sample and standard well positions. It is recommended to run all tests in duplicates.
4) Add rinsing buffer: add rinsing buffer, 50ul per well with multi-channel pipette.
5) **Add sample/standard/antibody**: add sample/standard into the wells, 50ul per each, then add bLF antibody, 50ul per well, shake gently and then cover the plate and incubate at 25 ◦C for 60min.
6) **Wash**: take out the plate and pour the liquid out. Use the diluted wash buffer (buffer 1) to wash the plate, 250ul/well. Wash for 4-5 times with interval of 10s. The pour the liquid out and tap the plate against absorbent paper. Eliminate the air bubble in the wells with micropipette tip if the bubble exists.
7) **Add enzyme conjugate**: add enzyme conjugate, 100ul per well, shake gently and then incubate at 25 ◦C for 30min. Then take out and repeat **Wash Step**.
8) **Coloration development**: add TMB substrate, 100ul per well, and then cover the plate and incubate at 25 ◦C for 15min.
9) **Stop the reaction**: add stop solution, 100ul per well, shake gently and read the plate with ELISA reader at 450nm.
10) Read the plate within 5min after adding stop solution.

9. Result Calculation

Qualitative estimation

With ELISA reader, a standard curve can be plotted with the ODs obtained. Use Logit-log, Cubic spline or logistic curve, etc to calculate the bLF sample content.
Usually these software will be installed with your ELISA reader. If it is not provided, please contact us for help, spreadsheet with Logit-log calculation will be provided upon your request.

10. Performance of the kit

1) Limit of quantification: 1.6 μg/ml
2) Specificity: 100% to bLF
3) Recovery: 80%-110%
4) Precision: C.V<10%.

11. Cautions and tips for the test

1) Return the ELISA kit and all reagents to room temperature (20-25◦C/68-77◦F). The incubation is 25 ◦C, lower or higher temperature will cause changes of OD and sensitivity of the kits, which may affect the result of the assay.
2) Wash step is vital for the reproducibility of the kit. Please wash according to the kit instruction. Do not let the plate dry during wash. Continue the next operations immediately after wash step.
3) Shake each reagent gently before use.
4) Stop solution is acidic, please handle with care.
5) Do not use expired kits and reagents. Do not mix the reagent and kits from different LOT.
6) The kit is stored at 2-8◦C(36-46◦F), do not freeze.
7) TMB substrate is sensitive to sunlight. Avoid direct sunlight.
8) The development step takes 15min. You can prolong it to 20min-25min if the color of the well is too light. On the contrary, please reduce the incubation time.

12. Storage and expiration

The kit is valid for 12months when stored at 2-8 ◦C. batch and Expiry information are printed on the package.