Bovine Lactoferrin ELISA Kit

bLF ELISA

Product #: E6001-96T

Manufacturer Information

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1. Description

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.

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

2. Application

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

3. Kit components

1) Microtiter plate, 96wells, 1 plate
2) bLF standards, 1mL/vial, 0, 0.1, 0.3, 0.9, 2.7, 8.1 μg/mL
3) HRP Enzyme conjugate, 12mL, with red cap
4) bLF Antibody, 7mL, with green cap
5) TMB Substrate, 12mL, with brown cap
6) Stop solution 8mL (0.5M sulfuric acid)
7) 20x Wash buffer, 50mL
8) 4x Sample buffer, 50mL

4. Instrument and material required

1) ELISA reader, with 450/630nm
2) Centrifuge
3) Balance, 0.01g
4) Centrifuge tube, 2mL, 10mL
5) Vortex mixer
6) Micropipette, 20-200ul, 100-1000ul
7) Multi-channel pipette, 250ul
8) graduated pipette,10ml

5. Reagent required

Deionized water

6. Buffer preparation

6.1 Buffer 1: wash buffer

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

This diluted wash buffer can be stored at 4°C for 1 month.

6.2 Buffer 2: sample buffer

Dilute 4x sample buffer with deionized water, in the volume ratio of 1:3, fox sample, 10mL 4x sample buffer + 30mL deionized water, mix thoroughly.

This diluted sample buffer can be stored at 4°C 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 / other sample

1) Take 0.1±0.005g sample into a new centrifuge tube, add 1ml sample buffer (Buffer 2), vortex for 1min to dissolve.

   Note: If bLF content in sample is high, please further dilute the sample with sample buffer to make sure the bLF content is within the range of the Standard Curve of this bLF ELISA Kit.

   For example, if the claimed bLF content of a sample is 4.5mg/g, then further dilute the dissolved sample with sample buffer to 1:1000, e.g., 1mL dissolved sample + 999mL sample buffer. Mix thoroughly.
2) Take 50ul per well for assay.

8. Notice and precautions before assay

1) Make sure the ELISA kit and all reagents are returned to room temperature (20-25 °C /68-77 °F). For example, keep these reagent and kits at room temperature for at least 60min.
2) Return unused kit components to 2-8 °C.
3) Washing step is important for the reproducibility of the kit, please follow this instruction carefully.
4) Cover the ELISA plate during all incubation. Avoid direct sunlight.

9. 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 60min.
2) Take needed microwells and zip rest in the zip-bag and return to 2-8 °C.
3) Layout the plate and record sample and standard well positions. It is recommended to run all tests in duplicates.
4) **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 37 °C /98.6 °F for 30min.
5) **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.
6) **Add HRP enzyme conjugate**: add enzyme conjugate, 100ul per well, shake gently and then incubate at 37 °C /98.6 °F for 30min. Then take out and repeat **Wash Step**.
7) **Coloration**: add TMB substrate, 100ul per well, and then cover the plate and incubate at 37 °C /98.6 °F for 15min.
8) **Stop the reaction**: add stop solution, 50ul per well, shake gently and read the plate with ELISA reader at 450nm. Read the plate within 5min after adding stop solution.

10. Result Calculation

10.1 Qualitative estimation

This kit is based on competitive ELISA, thus the OD values is inversely proportional to the bLF content contained in sample. If there is no ELISA reader, just compare the color of sample with the bLF Standard to get the estimated sample bLF content.

For example, average ODs of bLF standards (0, 0.1, 0.3, 0.9, 2.7, 8.1 μg/mL) are 2.000, 1.595, 1.050, 0.520, 0.220, 0.102, while sample 1# OD is 0.2, then the bLF content of the diluted sample will be within 2.7μg/mL to 8.1μg/mL. Further multiplied with the dilution factor, the real bLF in sample

can be estimated.

In this case, the color of sample 1# will be within the range of bLF Standard 5 and 6.

10.2 Quantitative calculation

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.

11. Sample dilution factor: 10

Please notice if the dissolve sample solution is further diluted with buffer 2, the new dilution factor shall be considered.

12. Specifications of the kit

1) Sensitivity: 0.1 mg/L
2) Specificity: 100% to bLF
3) Limit of Detection: 1mg/L
4) Recovery: 80%±20%
5) Precision: C.V<10%.

13. Cautions and tips for the test

1) Lower room temperature, e.g., lower than 20◦C may cause lower OD values. Please make sure all reagent and kit components are returned to room temperature.
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) If Standard 1 (0μg/mL) OD is lower than 0.5, please do not use. The kit may be expired or deteriorated.
9) The coloration 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.
10) The incubation is 37◦C /98.6◦F, lower or higher temperature will cause changes of OD and sensitivity of the kits, which may affect the result of the assay.

14. Storage and expiration

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