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# **Lactoferrin ELISA Kit**

| 1 Image |
|---------|
|---------|

2

**Publications** 



Go to Product page

## Overview

| Quantity:                | 96 tests               |
|--------------------------|------------------------|
| Target:                  | Lactoferrin (LTF)      |
| Reactivity:              | Cow                    |
| Method Type:             | Competition ELISA      |
| Detection Range:         | 3.12 μg/mL - 200 μg/mL |
| Minimum Detection Limit: | 3.12 μg/mL             |
| Application:             | ELISA                  |

## **Product Details**

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|--------------------|--|
| Purpose:           | For the quantitative determination of bovine lactoferrin (LTF/LF) concentrations in milk.  |
| Sample Type:       | Milk   |
| Analytical Method: | Quantitative   |
| Detection Method:  | Colorimetric   |
| Specificity:       | This assay has high sensitivity and excellent specificity for detection of bovine LTF. No significant cross-reactivity or interference between bovine LTF and analogues was observed. Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between bovine LTF and all the analogues, therefore, cross reaction may still exist. |
| Components:        | <ul><li>Assay plate</li><li>Standard</li></ul>   |

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• HRP-conjugate (100 x concentrate)

- · Sample Diluent
- HRP-conjugate Diluent
- Wash Buffer (25 x concentrate)
- TMB Substrate
- · Stop Solution
- · Adhesive Strip
- · Stop Solution
- · Adhesive Strip

## **Target Details**

| Target:             | Lactoferrin (LTF)   |
|---------------------|---|
| Alternative Name:   | lactotransferrin (LTF Products)   |
| Background:         | Abbreviation: LTF   |
|                     | Alias: GIG12, HLF2, LF, growth-inhibiting protein 12 lactoferrin neutrophil |
|                     | lactoferrin talalactoferrin   |
| UniProt:            | P24627  |
| Pathways:           | Transition Metal Ion Homeostasis  |
|                     |   |
| Application Details |   |
| Application Notes:  | Optimal working dilution should be determined by the investigator.          |

| Application Notes:   | Optimal working dilution should be determined by the investigator.                                     |
|----------------------|--|
| Sample Volume:       | 50 μL  |
| Assay Time:          | 1 - 4.5 h  |
| Plate:               | Pre-coated   |
| Protocol:            | 1. Prepare reagents, samples and standards as instructed.  |
|                      | 2. Set a Blank well without any solution.  |
|                      | 3. Add 50 µL standard or sample to each well.  |
|                      | 4. Add 50 μL HRP-conjugate (1x) to each well (Not to Blank well).                                      |
|                      | 5. Incubate 1 hour at 37 °C  |
|                      | 6. Aspirate and wash 5 times.  |
|                      | 7. Add 90 $\mu L$ of TMB Substrate to each well. Incubate for 20 minutes at 37 °C. Protect from light. |
|                      | 8. Add 50 $\mu$ L Stop Solution to each well. Read at 450 nm within 5 minutes.                         |
| Reagent Preparation: | 1. HRP-conjugate (1x) - Centrifuge the vial before opening. HRP-conjugate requires a 100-fold          |
|                      |  |

- dilution. A suggested 100-fold dilution is 10  $\mu$ L of HRP-conjugate + 990  $\mu$ L of HRP-conjugate Diluent.
- 2. Wash Buffer(1x)- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 mL of Wash Buffer (1 x).
- 3. Standard Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the Standard with 1.0 mL of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 5  $\mu$ g/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 150  $\mu$ L of Sample Diluent into each tube (S0-S6). Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (5  $\mu$ g/mL). Sample Diluent serves as the zero standard (0  $\mu$ g/mL).

#### Note:

- Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
- Bring all reagents to room temperature (18-25 °C) before use for 30 min.
- To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10 µL for once pipetting.
- Distilled water is recommended to be used to make the preparation for reagents.
   Contaminated water or container for reagent preparation will influence the detection result.

#### Sample Preparation:

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged toremove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determine compatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a
  possibility of causing a deviation due to the introduced chemical substance. The
  recommended dilution factor is for reference only.
- Please estimate the concentration of the samples before performing the test. If the values are not in therange of the standard curve, the optimal sample dilution for the particular experiment has to be determined. Samples should then be diluted with PBS (pH =7.0-7.2).

### Note:

For milk samples, recommend to determine the dilution factor by pretest. The optimal dilution factor should be determined by users according to their particular experiments. 5

#### Assay Precision:

Intra-assay Precision (Precision within an assay): CV%<8% Three samples of known concentration were tested twenty times on one plate to assess.

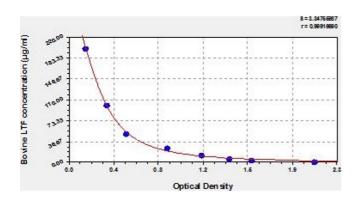
Inter-assay Precision (Precision between assays): CV%<10% Three samples of known

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## **Application Details**

| Application Details            |   |
|--------------------------------|---|
|                                | concentration were tested in twenty assays to assess.   |
| Restrictions:                  | For Research Use only   |
| Handling                       |   |
| Storage:                       | 4 °C,-20 °C   |
| Storage Comment:  Expiry Date: | Unopened kit Store at 2 - 8°C. Do not use the kit beyond the expiration date. May be stored for up to 1 month at 2 - 8°C. Coated assay Try to keep it in a sealed aluminum foil bag, plate and avoid the damp. Standard May be stored for up to 1 month at 2 - 8°C. If don't make recent use, better keep it store at HRP-conjugate -20°C. Opened kit HRP-conjugate Diluent Sample Diluent May be stored for up to 1 month at 2 - 8°C. Wash Buffer TMB Substrate Stop Solution *Provided this is within the expiration date of the kit. |
| Publications                   |   |
| Product cited in:              | Sharma, Huynh, Kim, Ghosh, Sodhi, Singh, Kim, Lee, Hussain, Oh, Jeong: "A PiggyBac mediated approach for lactoferricin gene transfer in bovine mammary epithelial stem cells for management of bovine mastitis." in: <b>Oncotarget</b> , Vol. 8, Issue 61, pp. 104272-104285, (2017) ( PubMed).  Sharma, Kim, Sodhi, Luong, Kim, Oh, Jeong: "Differentiation dynamics of mammary epithelial stem cells from Korean holstein dairy cattle under ECM-free conditions." in: <b>Journal of</b>  |

biomolecular structure & dynamics, pp. 1-22, (2015) (PubMed).



## **ELISA**

Image 1. Typical Standard Curve