

Bovine IgG ELISA Kit

Product #: E6012

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1. Description

Bovine milk contains many components that have immunomodulatory and antimicrobial properties. Three major classes of immunoglobulins are known to exist in cattle: IgG, IgM, and IgA. Bovine Immunoglobulin G (bovine IgG), as the main immunoglobulins in milk, plays important role in the immune system. In colostrum, bovine IgG is identified as the quality indicator. This ELISA Kit is based on double antibody sandwich ELISA to detect bovine IgG quantitatively in various dairy products in 75min.

2. Principle

This ELISA Kit is based on double antibody sandwich ELISA. A bovine IgG specific monoclonal antibody is pre-coated in the microwells, then IgG standard or Sample is added and form antibody-IgG complex, free IgG or sample will be removed after washing step, then HRP-IgG specific antibody is added to combine with the IgG captured on the wells. After washing, TMB substrate is added, the OD value of the sample is positively correlated to IgG in sample. A standard curve is plotted by regression and multiplied by the corresponding dilution factor to calculate IgG concentration in the sample.

3. Application

This kit is for quantitative determination of bovine IgG in milk and milk powder.

4. Kit components

- 1) Microtiter plate, 96wells, 1 plate
- 2) Bovine IgG standards x6 vials, 1mL/vial, 0, 0.05, 0.20, 0.60, 2.00, 8.00 µg/mL
- 3) Enzyme conjugated antibody, 12mL, with brown cap
- 4) TMB Substrate, 12mL, with brown cap
- 5) 2x Sample buffer, 50mL, with transparent cap
- 6) 2x Milk powder buffer, 50mL, with transparent cap
- 7) 20x Wash buffer, 50mL, with transparent cap
- 8) Stop solution, 7ml, with white cap
- 9) Plate cover, 2pcs

In case stop solution is not provided in the kit, please use 2M sulfuric acid as stop solution.

5. Instrument and material required

- 1) ELISA reader, with 450/630nm
- 2) Centrifuge
- 3) Balance, 0.0001g
- 4) Centrifuge tube, 2mL, 5mL, 15mL
- 5) Vortex mixer
- 6) Micropipette, 5-50µl, 20-200µl, 100-1000µl
- 7) Multi-channel pipette, 300µl
- 8) Incubator, 25°C

6. Reagent required but not provided

Deionized water or ultrapure water

7. Reagents preparation

Wash buffer:

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

This diluted wash buffer can be stored at 2-8°C for 1 month.

Sample buffer:

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

This diluted wash buffer can be stored at 2-8°C for 1 month.

Milk powder sample buffer:

Dilute 2x milk powder buffer with deionized water, in the volume ratio of 1:1, for example, 10mL 2x wash buffer + 10mL deionized water, mix thoroughly.

This diluted wash buffer can be stored at 2-8°C for 1 month.

Stop solution: 2M sulfuric acid, which should be prepared by customer themselves. The recipe can be provided upon request.

8. Sample preparation

8.1 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.

8.2 Raw milk

Dilute the milk with sample buffer in the volume ratio of 1:5000, and then take 100µl/well for analysis. **For example**, 20ul milk sample + 980ul sample buffer, mix thoroughly, then take 10ul mixed sample and dilute with 990ul sample buffer. This final mixed solution can be used for assay directly.

Further dilution may require if the IgG concentration in sample is over the Standard Curve range of the kit.

8.3 Milk powder

Take 1.0000 ± 0.0050 g milk powder sample into a new centrifuge tube (15mL), add 10ml deionized water, vortex thoroughly to dissolve it, then take 100µl of dissolved milk into a 2ml centrifuge tube, then add **900µl milk powder sample buffer**, vortex for 10s to mix, take 100µl/well for analysis.

Further dilution with deionized water may require if the IgG concentration in sample is over the Standard Curve range of the kit.

Note: in case the IgG level in diluted sample is not detected, please adjust the dilution factor accordingly.

Note: in case of running a recovery test, dilute the sample so that the IgG concentration is between 0.05 – 0.2 µg/mL.

9. 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 reagents and kits at room temperature for at least 30min.
- 2) Return unused kit components to 2-8 °C after use. Opened kit shall be used within 30days.
- 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.

10. Assay procedures

- 1) Take needed microwells and zip rest in the zip-bag and return to 2-8 °C.
- 2) Layout the plate and record sample and standard well positions. It is recommended to run all tests in duplicates.

- 3) **Add sample/standard:** add **sample/standard** into the wells, **100µl per well**, then cover the plate and incubate at **25 °C for 30min**.
- 4) **Wash:** take out the plate and pour the liquid out. Use the diluted wash buffer to wash the plate, **250µl/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 exists.
- 5) **Add enzyme conjugated antibody, 100µl per well**, shake gently and then cover the plate and incubate at **25 °C for 30min**.
- 6) **Wash:** take out the plate and pour the liquid out. Use the diluted wash buffer to wash the plate, **250µl/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 exists.
- 7) **Coloration:** add TMB substrate, **100µl per well**, and then cover the plate and incubate at **25 °C for 15min**.
- 8) **Stop the reaction:** add **stop solution, 50µl per well**, shake gently and read the plate with ELISA reader at 450nm. Read the plate within 5min after adding stop solution.

11. Result Calculation

This kit is based on sandwich ELISA, thus the OD values is proportional to the IgG contained in sample. With ELISA reader, a standard curve can be plotted with the ODs obtained. You can use the IgG standards to finish the standard curve, x-axis is the logarithm of the concentration of IgG standards, y-axis is the OD percentage of standards. Please note that the sample concentration you get from the standard curve is the diluted sample.

$$\text{OD Percentage (\%)} = \frac{B}{B_0} \times 100\%$$

B – mean OD of standards or samples

B₀ – mean OD of first standard (0 µg/ml)

12. Sample dilution factor

Raw milk, 5000

Bovine milk powder: 100

Note: If further dilution or customized dilution is performed in assay, please do multiply the dilution factor accordingly.

13. Limit of Detection (LOD)

Raw milk, 250 µg/ml

Bovine milk powder: 5 µg/g

Caution: the sample detection depends on the dilution factor, make sure you calculate the final result correctly.

14. Specifications of the kit

- 1) Sensitivity: 0.05 µg/ml
- 2) Specificity: 100% to bovine IgG, Lactoferrin<5%
- 3) Recovery: 80-120%
- 4) Precision: C.V<10%.

15. 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 (20-25 °C).
- 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. Do 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 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.

16. Storage and expiration

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