

ELISA for Lactoferrin

Apparatus and Equipment:

Analytical Balance

Microplate Reader

Tubes, 4 mL

Chemical Reagents:

Bovine Lactoferrin ELISA Qualification Set

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ELISA Starter Accessory Kit II

Reference Preparation:

- Reference Solution - Dilute 100 μ L of lactoferrin standard in 2.0 mL of dilution buffer. Do a 2nd dilution of 200 μ L of lactoferrin solution in 2.0 mL of dilution buffer. Do a 3rd dilution of 200 μ L of lactoferrin solution in 2.0 mL of dilution buffer.

Reference Preparation:

- Reference Solution - Dissolve 100 mg of reference lactoferrin in 50 mL of water. Dilute 100 μ L of lactoferrin reference solution in 4.0 mL of dilution buffer. Do a 2nd dilution of 200 μ L of lactoferrin solution in 4.0 mL of dilution buffer. Do a 3rd dilution of 200 μ L of lactoferrin solution in 4.0 mL of dilution buffer.

Sample Preparation:

- Sample Solution - Dissolve 100 mg of lactoferrin in 50 mL of water. Dilute 100 μ L of lactoferrin solution in 4.0 mL of dilution buffer. Do a 2nd dilution of 200 μ L of lactoferrin solution in 4.0 mL of dilution buffer. Do a 3rd dilution of 200 μ L of lactoferrin solution in 4.0 mL of dilution buffer.

Procedure:

1. Mix the antigen antibody with the coating buffer (1 μ L/100 μ L or 30 μ L/3.0 mL).
2. Add 100 μ L of the solution to each microwell.
3. Incubate at room temperature for 1 hour.
4. Wash plate 5 times.
5. Add 100 μ L of the blocking buffer to each microwell.
6. Incubate at room temperature for 30 minutes.
7. Wash plate 5 times.
8. Add 100 μ L of standard or sample solution to the microwells.
9. Incubate at room temperature for 1 hour.
10. Wash plate 5 times.
11. Add 100 μ L of diluted HRP detection antibody solution to the microwells.
12. Incubate at room temperature for 1 hour.
13. Wash plate 5 times.
14. Add 100 μ L of TMB Substrate Solution to each microwell.
15. Incubate in the dark at room temperature for 15 minutes.
16. Add 100 μ L of Stop Solution to each microwell.
17. Measure the absorbance of each microwell at 450 nm on a microplate reader.