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Mouse Anti-E. coli K99 IgG Antibody ELISA Kit

Product Number: ELK006

Shipping and Storage

1. Reagent kit storage: 2-8℃.
2. Validity period: 6 months.

Component

Component	96T
30× Concentrated Wash Buffer	20mL×1
Enzyme Conjugate	6mL×1
Microplate (Coated)	12 wells×8 strips
Sample Diluent	6mL×1
Chromogen Substrate A	6mL×1
Chromogen Substrate B	6mL×1
Stop solution	6mL×1
Positive control	0.5mL×1
Negative control	0.5mL×1

Description

This kit uses the double antigen sandwich method to determine the level of mouse Escherichia coli K99 antibody IgG (E.coli K99 IgG) in the specimen. Coat the microporous plate with purified antigen to prepare solid-phase antigen. Add E. coli K99 IgG antibody to the coated micropores in sequence, and then bind with HRP labeled antigen to form an antigen antibody enzyme labeled antigen complex. After thorough washing, add substrate TMB for color development. TMB is converted to blue under the catalysis of HRP enzyme and to the final yellow under the action of acid. The depth of color is positively correlated with the presence of E. coli K99 IgG antibody in the sample. Measure the absorbance (OD value) at a wavelength of 450nm using an enzyme-linked immunosorbent assay (ELISA) reader, and calculate the concentration of mouse Escherichia coli K99 antibody IgG (E.coli K99 IgG) in the sample using a standard curve.

Application

This kit is designed to measure the content of E. coli K99 IgG antibodies in mouse serum, plasma, and related liquid samples.

Testing Scope

1pg/mL-50pg/mL

Specimen requirements

1. Extract the specimen as soon as possible after collection, according to relevant literature, and conduct experiments as soon as possible after extraction. If the experiment cannot be conducted immediately, the specimen can be stored at -20℃, but repeated freezing and thawing should be avoided
2. Samples containing NaN3 cannot be detected because NaN3 inhibits the activity of horseradish peroxidase (HRP).

Protocol

1. Dilution of standard samples: This kit provides one original standard sample, and users can dilute it in a small test tube according to the following chart.

Standard 5	40pg/mL	Add 150μL of original standard to 150μL of standard dilution solution

Standard 4	20pg/mL	Add 150μL of standard 5 to a dilution of 150μL of standard solution
Standard 3	10pg/mL	Add 150μL of standard 4 to a dilution of 150μL of standard solution
Standard 2	5pg/mL	Add 150μL of standard 3 to a dilution of 150μL of standard solution
Standard 1	2.5pg/mL	Add 150μL of standard 2 to a dilution of 150μL of standard solution

2. Sample addition: Set up blank wells (blank control wells without sample or enzyme-linked immunosorbent assay, all other steps are the same), standard wells, and test sample wells. Accurately add 50μL of the standard sample on the enzyme-linked immunosorbent assay (ELISA) coated plate, first add 40μL of sample diluent to the well of the test sample, and then add 10μL of the test sample (the final dilution of the sample is 5 times). Add the sample to the bottom of the enzyme-linked immunosorbent assay plate well, avoiding touching the well wall as much as possible, and gently shake and mix well.
3. Incubation: Cover the plate with a sealing film and incubate at 37 ℃ for 30 minutes.
4. Solution preparation: Dilute 30 times the concentrated washing solution with distilled water and set aside for later use
5. Washing: Carefully remove the sealing film, discard the liquid, shake dry, fill each hole with detergent, let it stand for 30 seconds, then discard. Repeat this process 5 times and pat dry.
6. Enzyme addition: Add 50μL of enzyme labeled reagent to each well, except for blank wells.
7. Incubation: Follow the same procedure as in 3.
8. Washing: The operation is the same as 5.
9. Color development: Add 50μL of color developing agent A50 to each well, then add 50μL of color developing agent B50, gently shake and mix, and develop color at 37 ℃ in the dark for 10 minutes
10. Termination: Add 50μL of termination solution to each well to terminate the reaction (at this point, the blue color turns yellow).
11. Measurement: Zero the blank well and measure the absorbance (OD value) of each well in sequence at a wavelength of 450nm. The measurement should be conducted within 15 minutes after adding the termination solution.

Calculation

Draw a standard curve on a coordinate paper with the concentration of the standard substance as the horizontal axis and the OD value as the vertical axis, and determine the corresponding concentration based on the OD value of the sample from the standard curve; Multiply by the dilution factor again; Alternatively, the linear regression equation of the standard curve can be calculated using the concentration and OD value of the standard substance. By substituting the OD value of the sample into the equation, the sample concentration can be calculated, and then multiplied by the dilution factor to obtain the actual concentration of the sample.

Note

1. The kit should be balanced at room temperature for 15-30 minutes before being taken out of the cold storage environment. If the enzyme coated plate is not used up after opening, the Flat noodles should be stored in a sealed bag.
2. Concentrated washing solution may precipitate crystals. When diluting, it can be dissolved by heating in a water bath, and washing does not affect the results.
3. Each step of sample addition should use a sampler and its accuracy should be regularly checked to avoid experimental errors. It is best to control the sample addition time within 5 minutes. If there are a large number of specimens, it is recommended to use a sampling gun for sample addition.
4. Please make a standard curve at the same time as each measurement, preferably with a double hole. If the content of the substance to be tested in the specimen is too high (the OD value of the sample is greater than the OD value of the first well of the standard well), please dilute the sample diluent by a certain multiple (n times) before measuring. When calculating, please multiply by the total dilution multiple (×n ×5) at the end.
5. The sealing film is only for one-time use to avoid cross contamination.
6. Please store the substrate away from light.
7. Strictly follow the instructions for operation, and the judgment of the test results must be based on the reading of the enzyme-linked immunosorbent assay (ELISA) reader
8. All samples, detergents, and various waste materials should be treated as infectious agents.

9. Components from different batch numbers of this reagent must not be mixed.