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Human Rotavirus IgG Antibody ELISA Kit

Product Number: ELK12J1050

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
Standard (64ng/L)	0.5mL×1
Standard diluent	1.5mL×1

Description

This kit adopts the double-antigen sandwich method to determine the level of human Rotavirus Antibody IgG (RV IgG) in specimens. Purified human rotavirus antigen is coated on the microplate to prepare solid-phase antigen. Rotavirus Antibody IgG (RV IgG) is added sequentially into the coated microwells, followed by binding with HRP-labeled rotavirus antigen to form an antigen-antibody-enzyme-labeled antigen complex. After thorough washing, substrate TMB is added for color development. TMB is catalyzed by HRP to turn blue, and finally turns yellow under the action of acid. The color intensity is positively correlated with Rotavirus Antibody IgG (RV IgG) in the sample. The absorbance (OD value) is measured at a wavelength of 450 nm using a microplate reader, and the concentration of human Rotavirus Antibody IgG (RV IgG) in the sample is calculated via the standard curve.

Application

This kit is used for the determination of Rotavirus Antibody IgG (RV IgG) content in human serum, plasma and related liquid samples.

Testing Scope

1ng/L - 35ng/L

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	32ng/L	Add 150μL of original standard to 150μL of standard dilution solution

Standard 4	16ng/L	Add 150μL of standard 5 to a dilution of 150μL of standard solution
Standard 3	8ng/L	Add 150μL of standard 4 to a dilution of 150μL of standard solution
Standard 2	4ng/L	Add 150μL of standard 3 to a dilution of 150μL of standard solution
Standard 1	2ng/L	Add 150μL of standard 2 to a dilution of 150μL of standard solution

2. Sample addition: Set up blank wells (blank control wells receive no sample or enzyme conjugate; all other steps are identical), standard wells and test sample wells, Add 50μL of standard accurately to each standard well, For test sample wells: first add 40μL of sample diluent, then add 10μL of test sample (final sample dilution factor = 5). Dispense samples to the bottom of the wells, avoid touching the well walls, and mix gently by swirling.
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.