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Rat Toolans H1 Virus Antibody lgG ELISA

Product Number: ELK02J2099

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
Standard diluent	1.5mL×1

Description

This assay kit employs a double-antigen sandwich assay to quantify the expression of rat Toolan H1 IgG in specimens.A purified antigen is coated onto a microplate to create a solid-phase antigen, which can bind to rat Toolan H1 IgG in the sample. After washing to remove unbound antibodies and other components, the plate is incubated with HRP-labeled antigen to form an antigen–antibody–enzyme-labeled antigen complex. Following thorough washing, the substrate TMB is added to develop the color reaction.In the presence of HRP, TMB is converted to a blue color, and under acidic conditions, it is further converted to its final yellow form.Measure the absorbance (OD value) using a microplate reader at a wavelength of 450nm, and compare the result with the CUTOFF value to determine the presence or absence of rat Toolan H1 IgG in the specimen.

Application

This assay kit is designed to quantify the expression of Toolan H1 IgG in rat serum, plasma, and related liquid samples.

Specimen requirements

1. Extraction should be performed as soon as possible after specimen collection, following the protocols outlined in relevant literature; once extraction is complete, experiments should be conducted promptly.If immediate testing is not possible, the specimen may be stored at-20°C; however, repeated freeze-thaw cycles should be avoided.
2. Samples containing NaN3 cannot be detected, as NaN3 inhibits the activity of horseradish peroxidase (HRP).

Protocol

1. No.: Number the corresponding microwells of the samples sequentially; each plate shall include 2 negative control wells, 2 positive control wells, and 1 blank control well (the blank control well contains neither sample nor enzyme-labeled reagent; all other steps are performed identically).
2. Sample addition: Add 50μL of the negative control and the positive control to the negative and positive control wells, respectively.First, add 40μL of sample diluent to the test sample well, then add 10μL of the test sample.Add the sample to the bottom of the wells in the microplate, taking care not to touch the well walls, then gently shake the plate to mix the sample.

3. Incubation: Seal the plate with a sealing membrane and then incubate at 37℃ for 30 minutes.
4. Solution preparation: Dilute the 30× concentrated washing solution 30-fold with distilled water and set aside for later use.
5. Washing: Carefully remove the sealing membrane, discard the liquid, centrifuge to dry, then add washing buffer to each well, let it stand for 30 seconds, and discard; repeat this procedure five times, then blot dry.
6. Enzyme addition: Add 50μL of enzyme-labeled reagent to each well, except for the blank well.
7. Incubation: Perform as in step 3.
8. Rinse: Follow the procedure described in step 5.
9. Color development: Add 50μL of color development reagent A to each well, then add 50μL of color development reagent B; gently shake and mix thoroughly, and incubate at 37°C in the dark for 15 minutes.
10. Termination: Add 50μL of termination buffer to each well to terminate the reaction (the blue color will then turn yellow).
11. Measurement: Set the blank well to zero, then sequentially measure the absorbance (OD value) of each well at a wavelength of 450nm.The assay shall be performed within 15 minutes after adding the termination solution.

Calculation

Test validity: The mean value of the positive control wells shall be ≥1.00; the mean value of the negative control wells shall be ≤0.20.

Cut-off value calculation: Cut-off value = mean value of negative control wells + 0.15

Negative determination: A sample with an OD value <the cutoff value (CUT OFF) is considered negative for rat Toolan H1 IgG.

Positive determination: A sample is considered positive for rat Toolan H1 IgG if its OD value is ≥ the cutoff value (CUT OFF).

Note

1. Operate strictly in accordance with the instructions; do not mix components from different batch numbers of this reagent.
2. After being removed from a refrigerated environment, the assay kit should be equilibrated at room temperature for 15–30 minutes before use; if the enzyme-labeled microplate is not fully utilized after opening, the plate strips should be placed in an airtight bag for storage.
3. Crystallization may occur in the concentrated wash solution; when diluting, the solution can be warmed in a water bath to aid dissolution, and this procedure will not affect the experimental results.
4. The sealing film is for single-use only to prevent cross-contamination.
5. Store the substrate away from light.
6. The interpretation of test results must be based on the readings obtained from the microplate reader; when using dual-wavelength detection, the reference wavelength is 630nm.
7. All samples, washing solutions, and all waste materials shall be treated as infectious materials.The quenching solution is 2 M sulfuric acid; appropriate safety precautions must be observed during use.