Human Japanese Encephalitis IgG (JE IgG) ELISA
Cat No: K12-1586

Principle:

This is enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Japanese Encephalitis IgG (JE IgG) in samples. Addition of controls or sample to microtiter well which is pre-coated with human Japanese Encephalitis IgG (JE IgG) antibody, if Japanese Encephalitis IgG (JE IgG) antibody present, it will binds to the human Japanese Encephalitis IgG (JE IgG) antibody coated on plate during incubation. After washing addition of HRP conjugate to form immune complex. Unbound HRP conjugate will get removed by washing step after incubation. Then addition of Substrate A and B, develops blue color during incubation period and reaction will get stop after addition of stop solution with development of yellow color. The concentration of the Human AMA of sample is directly proportional to the yellow color developed in well and will be positively correlated.

Intended Use:

This kit is used for the qualitative detection of AMA in Human serum, blood plasma, and other related tissue Liquid.

Materials provided:

1. Microtiter Coated Plate (96 wells) – 1 no
2. Positive Control – 0.5 ml
3. Negative Control – 0.5 ml
4. HRP Conjugate – 6 ml
5. Wash Buffer (30X) - 20 ml
6. Sample Diluent – 6 ml
7. TMB Substrate A – 6 ml
8. TMB Substrate B – 6 ml
9. Stop Solution – 6 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. 37℃ incubator
2. Standard microplate reader.
3. Precision pipettes and Disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution
6. Absorbent paper.

Storage Information:

1. All reagents should be stored at 2 ºC to 8 ºC.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. 30 minutes prior before use, bring all components to room temperature (18-25 ºC). Store all the components of the kit at its appropriate storage condition after use.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Procedural Notes:

1. The kit takes out from the 2-8℃ environment, should be balanced 30 minutes in the room temperature then use. If the microelisa stripplate has not use up after opened, the plate should be stored in Sealed bag.
2. Add Sample with sampler Each step, And proofread its accuracy frequently, avoids the experimental error.
3. The operation carried out in strict accordance with instructions, test results must be based on microplate reader to determine readings shall prevail

4. In order to avoid cross-contamination, to avoid re-use in the hands of the suction head and seal plate membrane
5. Other agents do not have to be packaged or covered, This reagent which different batch number component do not mix.

Assay Procedure:

1. Set blank wells separately (blank wells don't add sample and HRP-conjugate reagent, other each step operation is same), Control wells, testing sample well. Add Diluted standard 50μl to standard well; Add Sample dilution 40μl to testing sample well which on Assay plate, then add testing sample 10μl (sample final dilute degree is 5 times), And Mixing gently shaking, incubated 30 minutes at 37℃..
2. Discard Liquid, drying, each well to fill 30-times diluted washing liquid, oscillation for 30 seconds, discard the washing liquid with absorbent paper Pat dry. Repeat five times, Pat dry.
3. Add HRP-conjugate reagent 50μl to each well, except the blank well. Mixing gently shaking, incubated 30 minutes at 37℃.
4. Discard Liquid, drying, each well to fill 30-times diluted washing liquid, oscillation for 30 seconds, discard the washing liquid with absorbent paper Pat dry. Repeat five times, Pat dry.
5. Add chromogen TMB solution A 50μl and chromogen TMB solution B 50μl to each well. Gently mix, incubate for 10 min at 37℃.Incubate at **37°C for 30 minutes**.
6. Add Stop Solution 50μl to each well, Stop the reaction (the blue color change to yellow color Immediately).
7. Take blank well as zero, measure the optical densit (OD) at 450 nm after adding Stop Solution and within 15 min

Interpretation of Results:

The validity of the experiment: the mean of the masculine comparative hole ≥1.00;
the mean of the feminine comparative hole ≤0.15;
The critical value calculation: the critical value=the mean of the feminine comparative hole+0.15;
Feminine determinant: if the sample OD value<the critical value, the Human (JE IgG) is feminine;
Masculine determinant: if the sample OD value≥the critical value, the Human (JE IgG) is masculine.

Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Performance Characteristics:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.