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Rat Kilham Virus IgG (KV-IgG)ELISA Kit

96 Tests

Catalogue Number: SLY1835Ra

Store all reagents at 2-8℃

Validity Period: six months

Method:Indirect ELISA

For samples:

In serum, plasma, culture media or any biological fluid.

FOR RESEARCH USE ONLY！

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS！

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING！

Rat Kilham Virus IgG (KV-IgG)ELISA Kit

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Purpose

Our Rat Kilham Virus IgG (KV-IgG)ELISA Kit is to for the qualitative determination of KV-IgG in Rat serum, plasma, culture media or any biological fluid.

Principle

The ELISA is based on the the qualitative enzyme immunoassay technique.The Microplate provided in this kit has been pre-coated with an antigen specific to KV-IgG, make it to solid-phase antigen.Samples are added to the Microplate wells and combined to the specific antigen. Then a Horseradish Peroxidase (HRP)-conjugated antigen specific for KV-IgG is added to each Microplate well and incubated,so the antigen-antibody-Enzyme labeled antigen complex is formed.Following a wash to remove any unbound reagent,then the TMB substrate solution is added to each well. Only those wells that contain KV-IgG and HRP conjugated KV antigen will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The qualitative determination of KV-IgG is determined by comparing with the CUT OFF value.

Sample collection and storages

Serum - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at approximately 3000×g. Remove serum and assay immediately or aliquot and store samples at -20℃ or -80℃.Avoid repeated freeze-thaw cycles

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 30 minutes at 3000×g at 2-8℃ within 30 minutes of collection. Store samples at -20℃or -80℃. Avoid repeated freeze-thaw cycles.

Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20℃or -80℃. Avoid repeated freeze-thaw cycles.

Note: The samples shoule be centrifugated dequately and no hemolysis or granule was allowed.

Materials required but not supplied

1. Standard microplate reader(450nm)
2. Precision pipettes and Disposable pipette tips.
3. 37 ℃ incubator

Precautions

1. Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer.

2. Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.

3. Mix all reagents before using.

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C)

Materials supplied

Name	96 determinations	48 determinations
Microelisa stripplate	12*8strips	12*4strips
Negative control	0.5ml	0.5ml
Positive control	0.5ml	0.5ml
HRP-Conjugate reagent	10.0ml	5.0ml
20X Wash solution	25ml	15ml
Sample Diluent	6.0ml	3.0ml
Chromogen Solution A	6.0ml	3.0ml
Chromogen Solution B	6.0ml	3.0ml
Stop Solution	6.0ml	3.0ml
Closure plate membrane	2	2
User manual	1	1
Sealed bags	1	1

Reagent preparation

20×wash solution:Dilute with Distilled or deionized water 1:20.

Assay procedure

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microelisa Stripplate.

2. Separately add Positive control and Negative control 50µl to the Positive and Negative well; Add testing sample 10µl then add Sample Diluent 40µl to testing sample well.

3. Add 100µl of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60 minutes at 37°C.

4. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Solution (400µl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add chromogen solution A 50µl and chromogen solution B 50µl to each well. Gently mix and incubate for 15 minutes at 37°C. **Protect from light.**

6. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not
appear uniform, gently tap the plate to ensure thorough mixing.

7. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

8. Determine the result

1. Test validity: the average of Positive control well≥1.00; the average of Negative control well ≤0.15.

2. Calculate Critical (CUT OFF): Critical= the average of Negative control well + 0.15.

Negative Result: sample OD< Calculate Critical (CUT OFF) is Negative.

Positive Result: sample OD≥ Calculate Critical (CUT OFF) is Positive.

Storage and validity

Storage: 2-8℃.

Validity: 6 months.