Human Rabies Virus antibody IgG ELISA Kit

Cat. No.: DEIA1027
Pkg. Size: 96T

Intended use

The Human Rabies Virus antibody IgG ELISA Kit is used for quantitative detection of IgG antibodies to Rabies Virus antibody in human serum or plasma. It is intended for diagnosing and monitoring of patients related to Rabies Virus.

General Description

The rabies virus is a neurotropic virus that causes fatal disease in human and animals. Rabies transmission can occur through the saliva of animals. The rabies virus has a cylindrical morphology and is the type species of the Lyssavirus genus of the Rhabdoviridae family. These viruses are enveloped and have a single stranded RNA genome with negative-sense. The genetic information is packaged as a ribonucleoprotein complex in which RNA is tightly bound by the viral nucleoprotein. The RNA genome of the virus encodes five genes whose order is highly conserved. These genes code for nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and the viral RNA polymerase (L). All transcription and replication events take place in the cytoplasm inside a specialized "virus factory", the Negri body (named after Adelchi Negri). These are 2–10 μm in diameter and are typical for a rabies infection and thus have been used as definite histological proof of such infection.

Principle Of The Test

The qualitative immunoenzymatic determination of antibodies against rabies virus is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with rabies virus antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material, horseradish peroxidase (HRP) conjugate is added. This conjugate binds to antigen-antibody complexes. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of rabies virus-specific antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

Reagents And Materials Provided

1. Human RV Ab Coated Wells, 96-well polystyrene microplate (12 strips of 8 wells): 1 plate
2. Human RV Standard, lyophilized recombinant human RV: 1 mL per bottle: 1 bottle
3. Assay Solution, 30 mL per bottle with preservatives: 1 bottle
4. Negative control, 1 mL per bottle: 1 bottle
5. HRP-CONJUGATE REAGENT, 12 mL per bottle with preservatives: 1 bottle
6. Wash Solution Concentrate (20x), 50 mL with preservatives: 1 bottle
7. CHROMOGEN SOLUTION A, 7 mL per bottle: 1 bottle
8. CHROMOGEN SOLUTION B, 7 mL per bottle: 1 bottle
9. Stop Solution, 7 mL per bottle: 1 bottle

Materials Required But Not Supplied

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Disposable V-shaped troughs.
5. Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
6. Absorbent tissue or clean towel.
7. Dry incubator or water bath, 37±0.5℃.
8. Microshaker for dissolving and mixing conjugate with samples.
9. Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
10. Microwell aspiration/wash system.

Storage
The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8 ℃, do not freeze and avoid light. To assure maximum performance of this rabies virus antibody ELISA kit, protect the reagents from contamination with microorganism or chemicals during storage.

Assay Steps
1. Allow the reagents and samples to reach room temperature (18-30°C) for at least 15-30minutes. Dilute the stock wash Buffer 1 to 20 with 1000ml of distilled or deionized water. Use only clean vessels to dilute the Wash buffer.
2. Diluting Sample: Dilute each sample 1:100 with sample diluent.
3. Adding Sample: Add 100μl of samples and 100μl Positive and Negative controls into their respective wells. Note: Use a separate disposal pipette tip for each specimen, Negative and Positive Controls as to avoid cross-contamination.
4. Sample Incubation: Cover the plate with the plate cover and incubate for 30minutes at 37°C. It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.
5. Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Washing buffer. Each time allow the microwells to soak for 30-60seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remaining liquids.
6. Adding HRP Conjugate: Add 100?μl of HRP-Conjugate Reagent into each well except for the Blank.
7. HRP-Conjugate Incubation: Cover the plate with the plate cover and incubate for 30minutes at 37°C.
8. Washing: Remove and discard the plate cover. Aspirate the liquid and rinse each well 5 times with Wash buffer (as step 5). After the final washing cycle, turn the strip plate and tap out any remainders.
9. Coloring: Add 50?μl of Chromogen A and 50?μl Chromogen B solution into each well including the Blank. Incubate the plate at 37°C for 15 minutes avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and anti-rabies virus positive sample wells.
10. Stopping Reaction: Using a multichannel pipette or manually, add 50?μl Stop solution into each well and mix gently. Intensive yellow color develops in Positive control and anti-rabies virus positive sample wells.
11. Measuring the Absorbance: Read the absorbance of each well at 450 nm within 10 minutes after adding the Stop Solution.

Calculation
Average the duplicate readings for each standard and sample and subtract the average zero standard optical density. 

Manual Plotting:
Plot on graph paper the absorbance of the standards against the standard concentration. Known concentrations of Human rabies virus are plotted on the X-axis and the corresponding absorbances on the Y-axis. The standard curve should result in a straight line that shows a direct relationship between Human rabies virus concentrations and the corresponding absorbances.
The concentration of Human rabies virus in samples may be determined by plotting the sample absorbances on the Y-axis, then drawing a horizontal line to intersect with the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding Human rabies virus concentration.

Note: Samples producing signals greater than that of the highest standard should be diluted in Assay Solution and reanalyzed. Multiply the measured concentration by the appropriate dilution factor.

**Plate Reader:**
An alternative approach is to use an ELISA curve fitting software. A linear curve plot is expected to produce the best fit of the resulting sample concentrations.

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**Precautions**

The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature (18-30 °C) before use. Shake reagent gently before, and return to 2-8 °C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37°C inside the incubator.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to rabies virus. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1 hour at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps for disposal.
18. The Stop solution (2M H2SO4) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. Proclin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.

20. Materials Safety Data Sheet (MSDS) available upon request.

21. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.