Technical Manual

Human Rabies Virus (HRV) IgG ELISA Kit

- Catalogue Code: HDES0125
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This kit is comprised by HRP conjugate, other reagents and ELISA Microtiter plate pre-coated with recombinant Rabies Virus (RBV) antigen. Apply the principle of enzyme-linked immunoassay (ELISA) to detect RBV-Ab in serum of human. During the experiment, add control and samples into the ELISA Microtiter plate, HRV-Ab will be bound with the antigen on the ELISA Microtiter plate. Then horseradish peroxidase (HRP) conjugate is added to each ELISA microtiter plate well, and substrate reagent is added for color development, the blue signal by Enzyme catalysis is in positive correlation of antibody content in sample. Measure the absorbance value of each well by using a microplate reader with 450 nm (630 nm) wavelength, then we can judge whether HRV antibody exist in the sample.

This assay employs the quantitative enzyme immunoassay technique. This kit is comprised by

The Human Anti-Rabies Virus IgG ELISA Kit detects and quantifies rabies virus-specific IgG in human serum or plasma of vaccinated, immunized and/or infected hosts. This immunoassay is suitable for:

- Determining immune status relative to non-immune controls;
- Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization and timing;
- Qualifying and standardizing vaccine batches & protocols.

For research use only (RUO), not for diagnosis, cure or prevention of the disease.

2. Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
HRP Conjugate (Red)	11 mL
Sample Diluent	25 mL
TMB Substrate	11 mL
Anti-Rabies Virus WHO international Standard	1 mL
Stop Solution	11 mL
20×Concentrated Wash Buffer	30 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece

Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

3. Other materials required but not supplied

- Micro-plate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
- High-precision transferpettor, EP tubes and disposable pipette tips
- 37° C Incubator or water bath
- Deionized water
- Absorbent paper

4. Notes

1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly performed. All the waste should be handled as contaminant.
2. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contacted carelessly.
3. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
4. Concentrated wash buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should keep fresh.
7. The results shall depend on the readings of the Microplate Reader.
8. **Each reagent is optimized for use in the HDES0125. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from otherHDES0125 with different lot numbers.**
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

Storage and expiry date

Store at 2-8°C. Avoid freeze.

Please store the opened plate at 2-8°C, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

5. Sample Collection and Storage

1. **Serum** Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
2. P**lasma** Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C.Avoid repeated freeze-thaw cycles.

6. Experimental preparation

Restore all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. **Sample preparation:** Dilute the serum or plasma samples with Sample Diluent (1:100) before test. The suggested 100-fold dilution can be achieved by adding 2μl sample to 198 μl of Sample Diluent.
2. **Wash Buffer:** The **10×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:9(10×Concentrated Wash Buffer (V): Deionized water (V) = 1:9). Wash Buffer can be conserved at 4°C in 1 week.
3. **Anti-Rabies Virus Standard**: Reconstitute the **Anti-Rabies Virus WHO international Standard**(NIBSC 19/244) with 1.0 mL **Sample Diluent**. Do not substitute other diluents. This reconstitution produces a stock solution of 4IU/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

   Dilution method: Take 6 EP tubes, add 500 μL of Reference Standard & Sample Diluent to each tube. Pipette 500 μL of the 4 IU/mL **Sample Diluent** to the first tube and mix up to produce a 2 IU/mL working solution. Pipette 500 μL of the solution from the former tube into the latter one according to this step. The illustration on the next page is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube. Gradient diluted standard working solution should be prepared just before use.

7. Assay procedure

Restore all reagents and samples to room temperature (25°C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8° C.

1. **Number:** number the standard, control or sample in order (multiple well), and keep a record of these wells. Set 2 wells for standard or control respectively. **(Samples need test in duplicate.)**
2. **Add sample:** add 100 µL of **standard** and **control** to standard control well, add 100 µL of **diluted Sample** to other sample well. Blow and mix fully.
3. **Incubate:** cover the plate sealer and mix thoroughly, incubate at 37°C for 1 hour in shading light.
4. **Wash:** remove the liquid in each well. Immediately add 300 µL of **Wash Buffer** to each well and wash. Repeat wash procedure for 3 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **HRP conjugate:** add 100 µL of **HRP Conjugate** into each well, cover the plate sealer and incubate at 37°C for 1 hour in shading light.
6. **Wash:** repeat step 4 for five times.
7. **Color Development:** add 100 µL of **Substrate Reagent** into each well, Cover the plate sealer and mix thoroughly, incubate at room temperature for 15 min in shading light.
8. **Stop reaction:** add 50 µL of **Stop Solution** into each well, mix thoroughly.
9. **OD Measurement:** Measure the absorbance value (A-value) of each well by using a Microplate Reader with 450 nm wavelength (use 630 nm as reference wavelength). **This step should be finished in 10 min.**

8. Result analysis

Average the duplicate readings for each standard and samples, Plot a four parameter logistic curve, with standard concentration on the x-axis and OD values on the y-axis.
If the OD value of the unknown sample is higher than the mean Optical Density value for the S6 standard, a precise quantification can not be performed. If a precise quantification is required, proceed to 1/10 dilution or more of the sample（The test concentration need to be multiplied by the dilution factor） and perform the assay again in order to have an Optical Density in the interval of the Standard curve.

Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.

9. Limitations

1. This kit is only used as the quantitative detection of RBV antibodies in serum of HUMAN. The antibody concentration can be calculated according to the values (International Unit, IU).
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com