Zhejiang AnyGo Technology Co., Ltd.
107-1, Area A, Building 1, No.6 Kaixuan South Road, Green Industry Cluster District, Quzhou City, Zhejiang, China.

Human Rabies Virus Protective Antibody ELISA Kit

Catalog Number: AK200H1

Package Size:96T

Intended use

The Human Rabies Virus Protective Antibody ELISA Kit is used for the semi-quantitative detection of human rabies virus protective antibody in serum or plasma.
For in vitro research use only.

Principle

Briefly, flat-bottom 96-well plates are coated with Glycoprotein of rabies virus (CVS-11strain) that captures rabies virus protective antibody in the sample. A quick wash removes any unbound particles. Captured rabies virus protective antibody is detected by Goat Anti-Human IgG pAb, which is conjugated to horseradish peroxidase (HRP). Finally, the chromogenic substrate 3, 3´, 5, 5´-tetramethylbenzidine (TMB) is added. The amount of rabies virus protective antibody is proportional to the color generated in the coupled oxidation-reduction reaction compared with known amounts of standards.

If You have Problems, please contact:

Phone: (+86) 010 84294548

Email: info@anygo-pet.com

Web: http://www.anygo-pet.com/

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

Kit Components

Reagents	Quantity	Description
96-well plate	8 wells × 12 strips	Coated with recombinant Glycoprotein (CVS11 strain) from CHO cells
Negative control (NC)	40 µL	Non-immunized serum from human
HRP conjugated pAb (100×)	120 µL	Goat Anti-Human IgG pAb
Enzyme dilution buffer	12 mL	20% calf serum-PBS buffer
Sample dilution buffer	12 mL	PBS-Triton X100
20×PBST buffer	50 mL	PBS-Tween 20
Stop buffer	6 mL	0.2M H_2SO_4
Substrate solution A	6 mL	H_2O_2
Substrate solution B	6 mL	Na Citrate buffer-TMB
Standard-1.5 IU/mL	40 µL	From EDQM
Standard-0.5 IU/mL	40 µL	From EDQM
Sealing films	2 pieces	/

Storage Conditions

The components of the kit remain stable through the expiration date indicated on the label and package when stored at 2-8 ℃, do not freeze and avoid light.

Materials Required (Not Supplied)

1. Water: freshly distilled or deionized
2. Disposable gloves, timer and appropriate waste containers.
3. Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
4. Microplate shaker or rotator（37±2℃）.
5. Microplate washer.
6. Vortex for dissolving and mixing conjugate with samples.
7. Microwell plate reader, single wavelength at 450nm or dual wavelength at 450nm and 620nm.

Protocol

1. Before using, pre-warm all the reagents to room temperature (18~30℃).

2. **Washing buffer** dilution: dilute the 20×PBST Buffer to distilled water at 1:20 (1ml 20× PBST+19ml H_2O) and mix well.

3. Dilute samples, negative control and standards (1.5 IU/mL,0.5 IU/mL) with **Sample Dilution Buffer** in ratio of 1:20 and mix well. For each sample, add 100μL into their respective wells in the pre-coated ELISA plate (Duplicated samples are recommended). Cover the plate with sealing films. Incubate with shaking at 37℃, 200 rpm for 60 minutes.

4. Empty the samples from wells and wash all wells with prepared **Washing Buffer** (350μL/well), repeat for 3 times. Remove residual solution with paper pat (optimal).

5. Dilute HRP-conjugated pAb with **Enzyme Dilution buffer** in ratio of 1:100 and mix well. Add 100μL of diluted HRP-conjugated pAb to each well. (Careful not to touch or scratch the surface of the wells). Incubate with shaking plate at 37℃, 200rpm for 60 minutes.

6. Remove samples from wells and wash all wells with prepared **Washing Buffer**(350μL/well), repeat for 3 times. Remove residual solution with paper pat (optimal).

7. Add 50μL each of Substrate Solution A and Substrate Solution B into each well. Mix thoroughly with shaking. Incubate at room temperature for 5 minutes and avoid light.

8. Stop the reaction by adding 50μL of Stop Solution into each well.

9. Read the absorbance at 450nm and 620nm (optimal) on a plate reader within 30 minutes after adding the Stop Solution.

Data Analysis

1. The OD value of negative control should be between 0.1 to 0.4.

2.Calculate the absorbance of OD450 or (OD450-OD620) for each standard and samples.

3.Campare results of Samples against the readings of Standard-1.5 IU/mL and Standard-0.5 IU/mL to estimate the VNA titer range of samples.

Properties

1. Precision: coefficient of variation CV (%) should not be higher than 15 % (n=10).

2. Stability: The components of the kit are left for 3 days at 37℃ under accelerated destruction condition, and all performance indexes still meet the above requirements.

3. Limit of detection: less than 0.2 IU/mL

Caution

A. Avoid cross contamination.

B. Follow reader measure as standard.

C. All samples and buffers are added or removed with pipette.

D. Do not mix reagents from different batches.