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Canine Rabies Virus Neutralizing Antibody ELISA Kit

Catalog Number: AK200D1

Package Size:96T

Intended use

The Canine Rabies Virus Neutralizing Antibody (VNA) ELISA Kit is used for the semi-quantitative detection of canine rabies virus neutralizing antibodies 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-11 strain) that captures rabies VNA in the sample. A quick wash removes any unbound materials. Captured rabies VNA is detected by Rabbit Anti-Canine IgG pAb conjugated with horseradish peroxidase (HRP). After wash, the chromogenic substrate 3, 3´, 5, 5´-tetramethylbenzidine (TMB) is added. The amount of rabies VNA is proportional to the color generated in the coupled oxidation-reduction reaction.

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 better service, please have the lot number of the kit ready when you contact us (found on the outside of the box).

Kit Components

Reagents	Quantity	Description
96-well plate	8 wells × 12 strips	Coated with recombinant Glycoprotein
Negative control	40 μL	Sera from non-rabies vaccine-immunized canines
Enzyme-conjugated pAb	120 μL	Rabbit Anti-Canine IgG pAb(1000X)
Enzyme dilution buffer	12 mL	20% calf serum-PBS buffer(not supplied)
Sample dilution buffer	12 mL	/
20×PBST buffer	50 mL	0.2M PBS-Tween 20
Stop buffer	6 mL	0.2M H_2SO_4(not supplied)
Substrate solution A	6 mL	/
Substrate solution B	6 mL	/
Standard-1.5 IU/mL	40 μL	CRIG standards diluted in negative canine sera
Standard-0.5 IU/mL	40 μL	CRIG standards diluted in negative canine sera
Sealing films	2 pieces	/

Storage Conditions

The components of the kit remain stable through the expiration date indicated on the label if 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 with temperature and speed control.

5. Microwell plate washer.

6. Vortex.

7. Microwell plate reader, single wavelength of measuring absorbance at 450nm or dual wavelength at 450nm and 620nm (correction wavelength).

8. Calf serum, 0.01M PBS (pH 7.2-7.6) and H_2SO_4

Protocol

1. Before using, pre-warm all the reagents to room temperature (18~30℃). It is recommended that all samples, controls, and standards be assayed in duplicates.

2. Buffer preparation:

Enzyme dilution buffer (20% CS-PBS): dilute Calf serum with PBS by 5-fold (for example:3 mL Calf serum+12 mL PBS) and mix well.

Washing buffer: dilute the 20×PBST Buffer with water by 20-fold and mix well.

Enzyme solution: Dilute Enzyme-conjugated pAb with **Enzyme Dilution buffer** 1:1000 (for example:12 μL Enzyme-conjugated pAb +12 mL **Enzyme Dilution buffer**) and mix well.

Stop solution: use 0.2M (or above) H_2SO_4 as the stop solution.

3. Dilute samples, negative control and standards (1.5 IU/mL,0.5 IU/mL) with **Sample Dilution Buffer** by 50-fold, mix well and transfer 100μL of each into their respective wells in the ELISA plate. Incubate at 37℃, 200 rpm for 60 minutes with constant shaking.

4. Remove samples from wells and wash all wells 3 times with **Washing Buffer** (250μL/well). Remove residual solution by taping against paper pat (optimal).

5. Add 100μL of **Enzyme** Solution (diluted Enzyme-conjugated pAb) to each well. (Careful not to touch or scratch the surface of the wells). Incubate plate at 37℃, 200rpm for 60 minutes with constant shaking.

6. Remove samples from wells and wash all wells 3 times with prepared **Washing Buffer** (250μL/well). Remove residual solution by taping against 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, mix well.

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

Data Analysis

1. The OD value of NC should be between 0.1 to 0.5.

2. Average the duplicate OD readings for each standards and samples.

3. Compare the results of Samples with Standard-1.5 IU/mL and Standard-0.5 IU/mL to confirm the titer range of samples.

Properties

1. Precision: coefficient of variation CV (%) less 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 0.1IU/mL.

Caution

A. Please read the instruction carefully prior to use.
B. Wear gloves and lab coats.
C. Avoid cross contamination.
D. Do not mix reagents from different batches.
E. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.