A167-EN.01

Rabies virus Glycoprotein G ELISA Kit
(For Vaccine Development)

Pack Size: 96 tests

Catalog Number：RAS-A167

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

INTENDED USE

This kit is developed for quantitative detection of Rabies virus Glycoprotein G in samples.

It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

Rabies virus (RABV), scientific name Rabies lyssavirus, is a deadly neurotropic virus that causes rabies in humans and animals. Rabies virus has an extremely wide host range and its transmission most often occur through the saliva of animals. Without intervention prior to disease progression, rabies has the highest case fatality of any infectious disease. RABV contains a single-stranded negative-sense R genome that encodes five structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and R-dependent R polymerase (L). Among these viral proteins, the RABV glycoprotein (RABV-G) is a pivotal player mediating virus entry and the major target of neutralizing antibodies, thus a key factor for vaccine and drug design. A rapid and effective assay kit detecting the levels of RABV-G is urgently needed to accelerate the development of RABV vaccines.

This assay kit is used to measure the levels of Glycoprotein G (RABV) by employing a standard sandwich-ELISA format. The microplate in the kit has been pre-coated with Anti-Glycoprotein G (RABV) Antibody. First add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the HRP-Anti-Glycoprotein G (RABV) Antibody to the plate, incubate and wash the wells. Lastly load the substrate into the wells and monitor color development in proportion with the amount of Glycoprotein G (RABV) present. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of Glycoprotein G (RABV) bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS167-C01	Pre-coated Anti-Glycoprotein G (RABV) Antibody Microplate	1 plate	Solid	2-8℃	2-8℃
RAS167-C02	Glycoprotein G (RABV) Standard	20 μg	Powder	2-8℃	-70℃

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RAS167-C03	HRP-Anti-Glycoprotein G (RABV) Antibody	20 μg	Powder	2-8℃	-70℃
RAS167-C04	10xWashing Buffer	50 mL	Liquid	2-8℃	2-8℃
RAS167-C05	2xDilution Buffer	50 mL	Liquid	2-8℃	2-8℃
RAS167-C06	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
RAS167-C07	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or dual wavelength microplate reader with 450nm and 630nm filter;

Centrifuge;

37ºC Incubator;

10 μL, 200 μL and 1000 μL precision pipettes;

10 μL, 200 μL and 1000 μL pipette tips;

Multichannel pipettes;

Tubes;

Graduated cylinder to prepare Wash Solution;

Deionized or distilled water to dilute 10× Washing Buffer;

STORAGE

1. Unopened kit should be stored at 2℃-8℃ upon receiving.

2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.

3. The opened kit should be stored per TABLE 1. The shelf life is 30 days from the date of opening.

REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in a 37 ºC incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

2. Reconstitute the provided lyophilized materials to stock solutions with distilled, sterile water as recommended in Table 2 and place the materials for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking. The reconstituted stock solutions should be stored at -70℃. It is recommended not to freeze-thaw

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more than 1 times, the packing specification shall not be less than 5 μg.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

ID	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
RAS167-C02	Glycoprotein G (RABV) Standard	20 μg	200 μg/mL	100 μL water
RAS167-C03	HRP-Anti-Glycoprotein G (RABV) Antibody	20 μg	200 μg/mL	100 μL water

RECOMMENDED SAMPLE PREPARATION

1. Working fluid preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

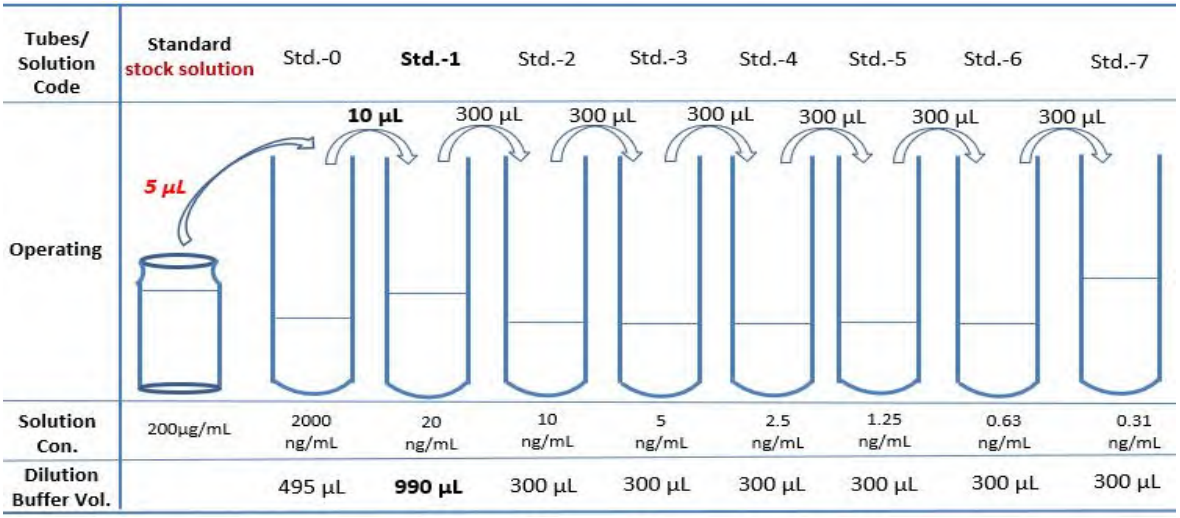
1.3 Preparation of HRP-Anti-Glycoprotein G (RABV) Antibody working fluid:

Dilute HRP-Anti-Glycoprotein G (RABV) Antibody to 0.2 μg/mL with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

2. Preparation of Standard curve

Make serial dilutions of the Glycoprotein G (RABV) as a Standard curve with Dilution Buffer as recommended in Figure 1.

FIGURE 1. PREPARATION OF 1:1 SERIAL DILUTIONS OF THE Glycoprotein G (RABV)



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3. Add Samples

Add 100μL serially diluted Glycoprotein G (RABV) Standard curve and samples to each well. For blank Control wells, please add 100μL 1×Dilution Buffer. Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

Note: *1. It is recommended to set multiple holes for samples and standard curves to be measured.*

2. If your test sample type is cell supernatant, the recommended minimum dilution of the sample MRD is 1:20.

4. Washing

Remove the remaining solution by aspiration, add 300 μL of 1×Washing Buffer to each well, gently tap the plate for 1 min, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

5. Add HRP-Anti-Glycoprotein G (RABV) Antibody

For all wells, add 100 μL **HRP-Anti-Glycoprotein G (RABV) Antibody (dilute to 0.2 μg/mL)** working solution. Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

6. Washing

Repeat step 4.

9. Substrate Reaction

Add 100 μL **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

10. Termination

Add 50 μL **Stop Solution** to each well, and tap the plate gently for 10 min to allow thorough mixing.

Note: *the color in the wells should change from blue to yellow.*

11. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer.

Note*: To reduce the background noise, subtract the value read at* $OD_{450\,nm}$ *with the value read at* $OD_{630\,nm}$.

CALCULATION OF RESULTS

1. Normal range of Standard curve: $R^2 \geq 0.9900$, detection range: 0.31-20 ng/mL.

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2. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.

3. To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is subtracted to the OD value of the blank control. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

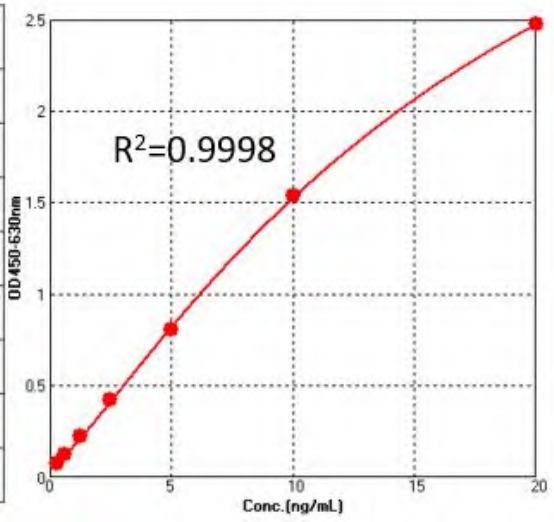
PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should be used according to the instructions.
3. Do not mix reagents from different lots.
4. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, warm to room temperature until the crystals have completely dissolved.
5. The kit should be stored at 2°C to 8°C.

TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only.

Glycoprotein G (RABV) Standard(ng/mL)	OD450-630nm
20	2.474
10	1.537
5	0.807
2.5	0.426
1.25	0.222
0.625	0.121
0.3125	0.075
0	0.029



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