T179-EN.01

Mouse Anti-RABV Nucleoprotein Antibody IgG Titer Serologic Assay Kit (ELISA)

Pack Size: 96 tests

Catalog Number： RAS-T179

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

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedure

INTENDED USE

The kit is developed for titer measurement of Anti-RABV Nucleoprotein Antibody IgG in mouse serum. 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 RNA genome that encodes five structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA-dependent RNA 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.

This assay kit is used to measure the titer of Anti-RABV Nucleoprotein Antibody IgG by employing an indirect ELISA. Immobilize RABV Nucleoprotein on the microplate. Then add the samples, incubate and wash the wells. Next add Secondary antibody HRP-Conjugated 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 antibody 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 antibody bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS179-C01	Pre-coated RABV Nucleoprotein Microplate	1 plate	Solid	2-8°C	2-8°C
RAS179-C02	RABV-N Antibody Positive Control	50 µL	Liquid	2-8°C	2-8°C
RAS179-C03	RABV-N Antibody Negative Control	50 µL	Liquid	2-8°C	2-8°C
RAS179-C04	HRP-Conjugated Antibody	50 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS179-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS179-C06	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C

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RAS179-C07	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
RAS179-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

STORAGE AND VALIDITY INSTRUCTIONS

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.

MATERIALS REQUIRED BUT NOT PROVIDED

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

Centrifuge;

37 ºC Incubator;

Single channel or multichannel pipettes with 10 µL, 200 µL and 1000 µL precision;

10 µL, 200 µL and 1000 µL pipette tips;

Test Tubes;

Graduated cylinder;

Deionized or distilled water for dilution;

REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) before use.

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 RABV-N Antibody Positive Control and RABV-N Antibody Negative Control working fluid and pre-treatment of samples:

a. For qualitative detection of antibodies:

Dilute the Samples, RABV-N Antibody Positive Control and RABV-N Antibody Negative Control at 1:100 with Dilution Buffer.

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b. For determination of antibody titer:

It is recommended to dilute the samples, RABV-N Antibody Positive Control and RABV-N Antibody Negative Control from 1:800-1:25600 with Dilution Buffer.

2. Plate set up

Number the diluted samples corresponding to the wells of the Pre-coated RABV Nucleoprotein Microplate. Each experiment requires a set of Positive Control and Negative Control working fluid.

3. Add Samples

Add 100 μL diluted Samples, Positive Control and Negative Control working fluid to the corresponding wells. Add 100 μL Dilution Buffer to blank control. Seal the plate with microplate sealing film and incubate at 37℃ for 1.0 h.

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-Conjugated Antibody

Dilute HRP-Conjugated Antibody solution at 1:2000 with Dilution Buffer to make a working solution. The prepared working fluid should be stored away from light.

For all wells, add 100 μL HRP-Conjugated Antibody working solution. Seal the plate with microplate sealing film and incubate at 37℃ for 1.0 h, avoid light.

6. Washing

Repeat step 4.

7. Substrate Reaction

Add 100 μL **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at 37℃ for 20 min, avoid light.

8. Termination

Add 50 μL **Stop Solution** to each well, and tap the plate gently for 3 min to allow thorough mixing.

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

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9. 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 $OD_{630\ nm}$.*

CUT-OFF VALUE IDENTIFICATION

Cut-off value =0.1

Normal range of Negative control (1:100): $OD_{450\ nm}$-$OD_{630\ nm}$< 0.1

Normal range of Positive control (1:800): $OD_{450\ nm}$-$OD_{630\ nm}$≥ 1.5

***Note：** The cut-off value can be determined by the end user.*

INTERPRETION OF RESULTS

a. For qualitative detection of antibodies:

Positive reading: $OD_{450\ nm}$-$OD_{630\ nm}$ of sample ≥ Cut-off value means Anti-RABV-N Antibody IgG are detected.

Negative reading: $OD_{450\ nm}$-$OD_{630\ nm}$ of sample < Cut-off value means Anti-RABV-N Antibody IgG are not detected.

b. For determination of antibody titer:

Determination of antibody titer: the positive sample was diluted with a gradient, and the antibody titer of the sample corresponds to the highest dilution factor that still yields a positive reading.

LIMITATIONS OF THE PROCEDURE

The kit cannot be used for quantitative detection.

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. This kit should be used according to the provided 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 the buffer solution, incubate until the crystals have completely dissolved. Before use, bring the solution back to room temperature.
5. This kit should be stored at 2°C -8°C.
6. Please prepare the working solution of each component according to the needs of the experiment, all prepared working solution is for one-time use and cannot be stored.

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TYPICAL DATA

Note: *The Typical data is for reference only.*

a. For qualitative detection of antibodies:

Value Result in units	Result	Test Result Interpretation
$OD_{450\ nm}$ - $OD_{630\ nm}$ =0.059	Negative	Anti-RABV-N Antibody IgG are not detected
$OD_{450\ nm}$ - $OD_{630\ nm}$ =0.427	Positive	Anti-RABV-N Antibody IgG are detected

b. For determination of antibody titer:

Note: *Quality control data between different plates should not be mixed, and negative and positive controls should be set for each test.*

Ratio of Dilution	$OD_{450\ nm}$ - $OD_{630\ nm}$ (**Samples**)	Result
100	3.115	The titer level of antibody is 25600
200	3.016	
400	2.897	
800	2.454	
1600	1.541	
3200	0.803	
6400	0.453	
12800	0.213	
25600	**0.136**	
51200	0.068	
Blank	0.012	

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