RA242-EN.01

Rabies Virus Nucleoprotein (strain ERA) ELISA Kit

Pack Size: 96 tests

Catalog Number: RAS-A242

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 specific quantitative detection of Rabies virus Nucleoprotein (strain ERA) in samples.

It is intended for research use only (RUO).

【Assay Principle】

Rabies virus (RABV), scientific name Rabies lyssavirus, is a deadly neurotropic virus that causes rabies in humans and animals. Rabies viruses have an extremely wide host range, and its transmission most often occurs through the saliva of animals. Without intervention prior to disease progression, rabies has the highest case fatality rate of any infectious disease. RABV possesses 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). A rapid and effective kit for quantifying RABV-N is urgently needed to accelerate the development of RABV vaccines.

This kit quantifies Rabies virus Nucleoprotein (strain ERA) using a sandwich ELISA format. The microplate is pre-coated with an Anti-Nucleoprotein (RABV) Antibody, which captures Rabies virus Nucleoprotein (strain ERA) present in standards and samples. After washing, an HRP-Anti-Nucleoprotein (RABV) Antibody is added to bind the captured Rabies virus Nucleoprotein (strain ERA), forming an Antibody-antigen-HRP-antibody sandwich complex. Following additional washes, a substrate is added for color development. The reaction is stopped with stop solution, and the color changes from blue to yellow. Absorbance is measured at 450 nm with a 630 nm reference. The absorbance signal is directly proportional to the Rabies virus Nucleoprotein (strain ERA) concentration in the sample.

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【Material Provided】

Table 1. Materials provided

Catalog	Components	Size (96 T)	Format	Storage	
				Unopened	Opened
RAS242-C01	Pre-coated Anti-Nucleoprotein (RABV) Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RAS242-C02	Rabies virus Nucleoprotein (strain ERA) Standard	4 μg×2	Powder	2-8°C	-70°C
RAS242-C03	HRP-Anti-Nucleoprotein (RABV) Antibody	20 μg	Powder	2-8°C, avoid light	-70°C, avoid light
RAS242-C04	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS242-C05	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS242-C06	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS242-C07	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

【Reagents and Consumables / Equipment Required but not Provided】

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

Timer;

37ºC Incubator;

10 μL, 200 μL, and 1000 μL precision pipettes;

10 μL, 200 μL, and 1000 μL pipette tips;

Multichannel pipettes;

Tubes;

Reagent bottle;

Deionized or distilled water to dilute 10×Washing Buffer;

【Storage】

1. Store the unopened kit at 2-8°C upon receipt.
2. Locate the expiration date on the outer packaging and do not use reagents beyond their expiration date.

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3. The opened kit should be stored according to the storage conditions listed in the Components Table. The shelf life of the opened kit is 30 days from the date of opening.

【Reagent Preparation】

Bring all reagents to room temperature (20-25°C) before use. If crystals are present in the solution, allow the reagents to equilibrate until the crystals are completely dissolved. If needed, incubate at 37°C for 10-15 minutes to facilitate dissolution.

According to Table 2, reconstitute the provided lyophilized product with ultrapure water to prepare the stock solution. Allow the vial to stand at room temperature for 15 to 30 minutes, then gently pipette up and down to mix. Do not vortex or shake vigorously.

Store the reconstituted stock solution at -70°C. It is recommended to aliquot the stock solution to avoid repeated freeze-thaw cycles. Do not exceed one freeze-thaw cycle. Each aliquot (RAS242-C02) should contain at least 2 μg of Standard, and each aliquot (RAS242-C03) should contain at least 5 μg of Antibody.

Table 2. Preparation method

ID	Components	Size	Concentration	Reconstitution Buffer and Vol.
RAS242-C02	Rabies virus Nucleoprotein (strain ERA) Standard	4 μg	20 μg/mL	200 μL water
RAS242-C03	HRP-Anti-Nucleoprotein (RABV) Antibody	20 μg	100 μg/mL	200 μL water

【Assay Procedure】

1. Preparation of Working Solution

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL of 10×Washing Buffer with ultrapure water/deionized water to a final volume of 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL of 2×Dilution Buffer with 1×Washing Buffer to a final volume of 100 mL.

1.3 Preparation of HRP-Anti-Nucleoprotein (RABV) Antibody working solution:

Dilute HRP-Anti-Nucleoprotein (RABV) Antibody to 0.4 μg/mL with 1×Dilution Buffer. The prepared working solution should avoid light. Please prepare it for one-time use only.

2. Preparation of the Standard

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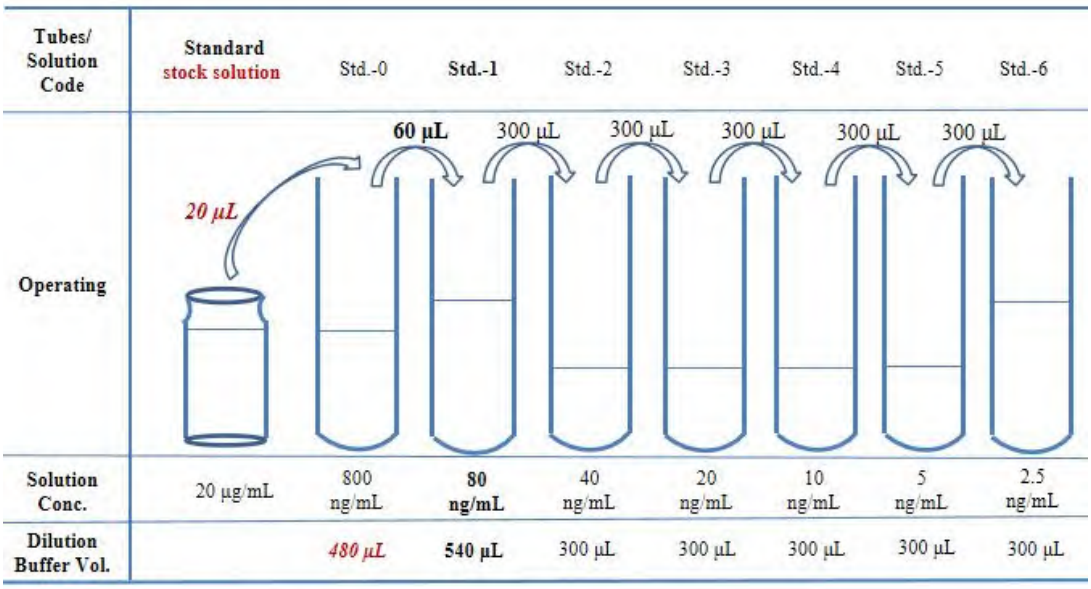
Prepare serial dilutions of the Rabies virus Nucleoprotein (strain ERA) standard using 1×Dilution Buffer as recommended in Figure 1.

Figure 1. Preparation of 2-fold serial dilutions of the Rabies virus Nucleoprotein (strain ERA) Standard



3. Addition of Samples

Add 100 µL serially diluted **Rabies virus Nucleoprotein (strain ERA)** Standard and samples to the appropriate wells. Add 100 µL of 1×Dilution Buffer to the Blank control wells. Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

Note: It is recommended to set double holes for samples and standard curves to be tested. It is recommended to use the example plate layout shown in Figure 2 to record the positions of standards and samples.

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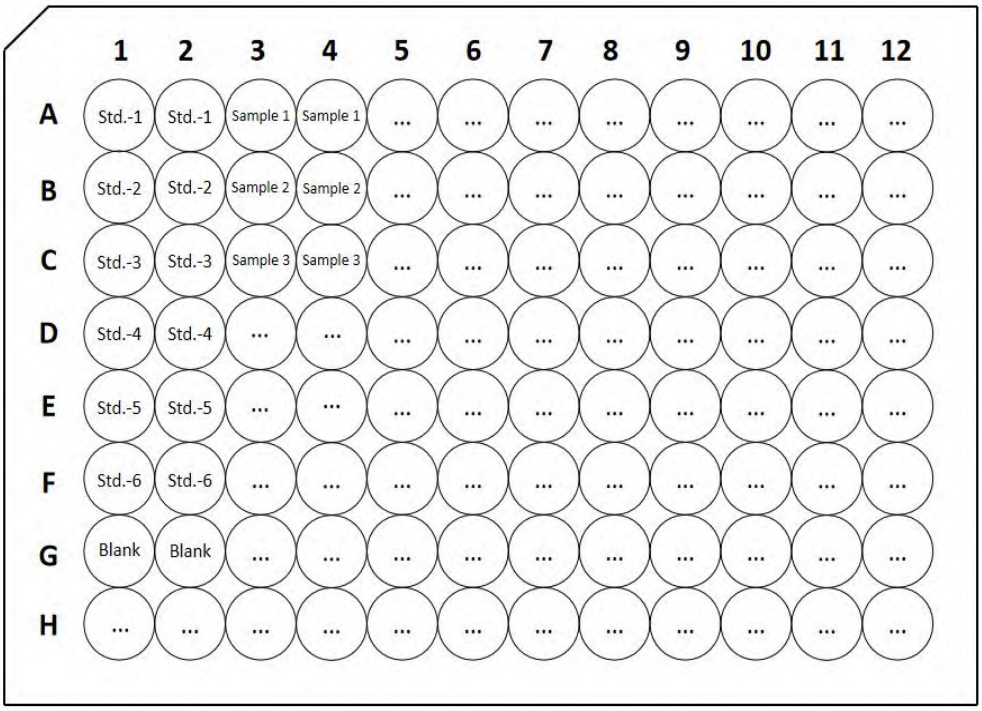
Figure 2. Recommended plate layout for standards and samples



4. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 30 s, remove any remaining 1×Washing Buffer by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the washing step above for three times.

5. Addition of HRP-Anti-Nucleoprotein (RABV) Antibody

Add 100 µL HRP-Anti-Nucleoprotein (RABV) Antibody working solution (0.4 µg/mL) to each well. Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

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 room temperature for 20 minutes, avoid light.

8. Termination

Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.

Note: The color in the wells will change from blue to yellow.

9. Data Recording

Measure the absorbance at 450 nm with a 630 nm reference within 5 minutes after adding the stop solution.

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Note: Subtracting the OD630nm value from the OD450nm value helps reduce background interference.

【Calculation of Results】

1. Calibrate absorbance values by subtracting the blank control OD from standard and test sample OD values. 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.
2. Normal range of Standard curve: $R^2 \geq 0.9900$, detection range: 2.5-80 ng/mL.
3. If the OD value of the sample to be tested is higher than that of the highest standard, the sample should be diluted with 1×dilution buffer, and the assay repeated.

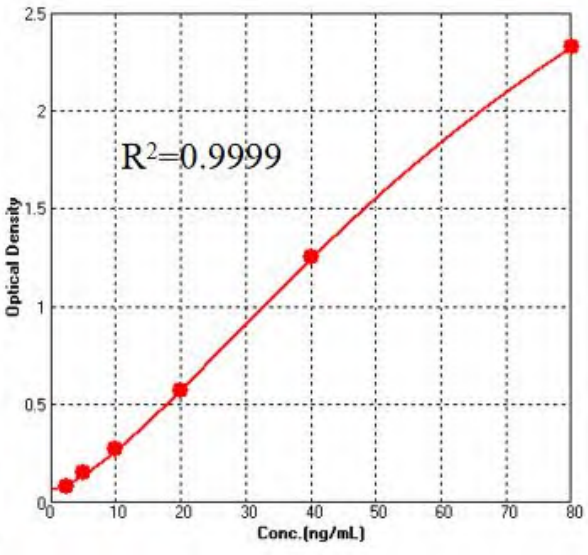
【Precautions】

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. All reagents should be balance to room temperature (20-25°C) 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 2-8°C.

【Typical Data】

The following data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Standard (ng/mL)	O.D.-1	O.D.-2	Average	Corrected
80	2.323	2.438	2.381	2.325
40	1.308	1.305	1.307	1.251
20	0.609	0.641	0.625	0.569
10	0.314	0.339	0.327	0.271
5	0.205	0.210	0.208	0.152
2.5	0.154	0.123	0.139	0.083
0	0.056	0.057	0.056	/



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【Precision】

1. Intra-assay Precision

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision.

2. Inter-assay Precision

Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (ng/mL)	60.654	30.582	4.967	61.396	30.627	4.791
SD	3.679	0.919	0.173	5.703	3.160	0.164
CV (%)	6.1	3.0	3.5	9.3	10.3	3.4

【Recovery】

Three samples with different concentrations were tested to calculate the recovery rate.

Sample(n=5)	Average Recovery %	Range %
High	94.7	86.4-102.3
Middle	101.0	91.0-113.0
Low	104.0	90.7-112.9

【Linearity】

To assess the linearity of the assay, samples spiked with high concentrations were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture medium (DMEM)	Cell culture medium (RPMI 1640)
1:2	Average Recovery (%)	101.5	96.5
	Range (%)	90.5-109.2	88.8-103.7
1:4	Average Recovery (%)	96.9	95.6
	Range (%)	94.1-99.6	93.1-98.1
1:8	Average Recovery (%)	100.9	98.3

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	Range (%)	92.7-107.8	94.2-101.6
1:16	Average Recovery (%)	103.9	104.2
	Range (%)	97.1-112.8	93.6-114.1

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【Troubleshooting Guide】

Problem	Cause	Solution
Low signal	a. Kit components were not equilibrated to room temperature before use; b. Insufficient reconstitution time for lyophilized components.	a. Remove the kit from 2-8 °C storage in advance and allow all reagents to fully equilibrate to room temperature before starting the assay; b. After adding reconstitution buffer, allow lyophilized components to stand for at least 15 minutes and mix gently before use.
Poor assay reproducibility	a. Improper storage of reagents after opening; b. In consistent timing during sample dilution of pipetting.	a. Store reagents strictly according to the instructions in this manual; b. Plan the experimental workflow in advance and properly schedule assay timing.
High background	a. Insufficient washing; b. Excessive incubation temperature or prolonged substrate development time; c. Reagent contamination.	a. Increase soak time during wash steps and ensure the plate is thoroughly blotted dry after final wash; b. Strictly follow the operating procedures described in the manual; c. Use clean reagents and consumables, and maintain a clean experimental environment.
Edge effects	Uneven temperature distribution across the plate.	Ensure uniform incubation temperature and avoid stacking plates during incubation.
Good standard curve but no detectable signal in samples	a. Interfering substances present in the samples; b. Target analyte concentration below the assay detection.	a. Optimize sample dilution to minimize matrix interference; b. Use a higher-sensitivity assay if lower analyte levels are expected.

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