T245-EN.01

Mouse Anti-Influenza A/Hong Kong/483/97 HA Antibody IgG Titer ELISA Assay Kit (HA)

Pack Size: 96 tests

Catalog Number: RAS-T245

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

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

INTENDED USE

The kit is developed for titer measurement of Anti-Influenza A (Hong Kong/483/97) HA antibody IgG in Mouse serum. It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

Influenza, commonly known as 'the flu', is an infectious disease of birds and mammals caused by RNA viruses of the family Orthomyxoviridae, the influenza viruses. The virus is divided into three main types (Influenza virus A, Influenza virus B, and Influenza virus C), which are distinguished by differences in two major internal proteins (hemagglutinin (HA) and neuraminidase (NA), which are the most important targets for the immune system. Hemagglutinin binds to the sialic acid-containing receptors on the surface of host cells during initial infection and at the end of an infectious cycle which makes it a great target for vaccine studies.

This assay kit is used to measure the titer of Anti-Influenza A (Hong Kong/483/97) HA antibody IgG by employing an indirect ELISA. Immobilize Influenza A (Hong Kong/483/97) HA 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.

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MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS245-C01	Pre-coated Influenza A (Hong Kong/483/97) HA Microplate	1 plate	Solid	2-8°C	2-8°C
RAS245-C02	Influenza A (Hong Kong/483/97) HA Antibody Positive Control	100 µL	Liquid	2-8°C	2-8°C
RAS245-C03	Influenza A (Hong Kong/483/97) HA Antibody Negative Control	100 µL	Liquid	2-8°C	2-8°C
RAS245-C04	HRP-Conjugated Antibody	50 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS245-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS245-C06	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS245-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS245-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

Note: *It is recommended that HRP-Conjugated Antibody be centrifuged briefly before use to deposit liquid from the tube wall or cap to the bottom of the tube.*

STORAGE AND VALIDITY INSTRUCTIONS

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.

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.

MATERIALS REQUIRED BUT NOT PROVIDED

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

Centrifuge;

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

a. For qualitative detection of antibodies:

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

b. For determination of antibody titer:

It is recommended to dilute the samples, Positive Control from 1:1600-1:102400 with Dilution Buffer, Negative Control at 1:100 with Dilution Buffer.

2. Plate set up

Number the diluted samples corresponding to the wells of the Pre-coated Influenza A (Hong Kong/483/97) HA Microplate. Each experiment requires a set of Positive Control and Negative Control working solution.

3. Add Samples

Add 100 µL diluted Samples, Positive Control and Negative Control working solution 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

Carefully remove the plate sealer and discard the liquid from the wells. Add 300 µL of 1×Washing Buffer to each well

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and soak for 30 seconds. Aspirate or decant the buffer. Repeat for a total of three washes. After the final wash, invert the plate and blot dry on absorbent paper.

5. Add HRP-Conjugated Antibody

Dilute **HRP-Conjugated Antibody** solution at 1:1000 with Dilution Buffer to make a working solution. The prepared working solution 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 minutes, avoid light.

8. Termination

Add 50 µL **Stop Solution** to each well, and tap the plate gently for 5 minutes to allow thorough mixing.

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

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 (1:1600): $OD_{450\ nm}$-$OD_{630\ nm}$>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-Influenza A (Hong Kong/483/97) HA

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antibody IgG are detected.

Negative reading: $OD_{450\ nm}$-$OD_{630\ nm}$ of sample < Cut-off value means Anti-Influenza A (Hong Kong/483/97) HA 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°C-25°C) 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 solutions are for one-time use and cannot be stored.

TYPICAL DATA

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

a. For qualitative detection of antibodies:

Value Result in units	Result	Test Result Interpretation
$OD_{450\ nm}$ - $OD_{630\ nm}$ =0.061	Negative	Anti-Influenza A (Hong Kong/483/97) HA antibody IgG are not detected
$OD_{450\ nm}$ - $OD_{630\ nm}$ =0.553	Positive	Anti-Influenza A (Hong Kong/483/97) HA antibody IgG are detected

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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
400	3.245	The titer level of antibody is 102400
800	3.051	
1600	2.901	
3200	2.039	
6400	1.247	
12800	0.697	
25600	0.409	
51200	0.240	
102400	0.127	
204800	0.084	
Blank	0.025	

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