

Mouse Anti-Influenza (A/Wisconsin/67/2022) Antibody IgG Titer ELISA Assay Kit (HA)

Pack Size: 96 tests

Catalog Number: RAS-T226

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-Influenza (A/Wisconsin/67/2022) 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/Wisconsin/67/2022) HA Antibody IgG by employing an indirect ELISA. Immobilize Influenza A (Wisconsin/67/2022) 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.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS226-C01	Pre-coated Influenza A (Wisconsin/67/2022) HA Microplate	1 plate	Solid	2-8°C	2-8°C
RAS226-C02	Influenza A (Wisconsin/67/2022) HA Antibody Positive Control	100 µL	Liquid	2-8°C	2-8°C
RAS226-C03	Influenza A (Wisconsin/67/2022) HA Antibody Negative Control	100 µL	Liquid	2-8°C	2-8°C
RAS226-C04	HRP-Conjugated Antibody	50 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS226-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS226-C06	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS226-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS226-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. Unopened kit should be stored at 2°C-8°C 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 °C Incubator;

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

10 μ L, 200 μ L and 1000 μ L pipette tips ;

Test Tubes ;

Graduated cylinder;

Deionized or distilled water for dilution;

REAGENT PREPARATION

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

RECOMMENDED SAMPLE PREPARATION

1. Working fluid preparation

1.1 Preparation of 1 \times Washing Buffer:

Dilute 50 mL 10 \times 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 1:50 with Dilution Buffer.

b. For determination of antibody titer:

It is recommended to dilute the samples, Positive Control from 1:1600-1:51200 with Dilution Buffer, Negative Control from 1:50 with Dilution Buffer.

2. Plate set up

Number the diluted samples corresponding to the wells of the Pre-coated Influenza A (Wisconsin/67/2022) HA Microplate. Each experiment requires a set of Positive Control and Negative Control working fluid.

3. Add Samples

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

4. Washing

Remove the remaining solution by aspiration, add 300 μ L of 1 \times Washing Buffer to each well, soak for 30s, remove any remaining 1 \times 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 1:1000 with Dilution Buffer to make a working solution. The prepared working fluid should be stored away from light.

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

6. Washing

Repeat step 4.

7. Substrate Reaction

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

8. Termination

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

Note: the color in the wells should 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 (1:50): OD_{450 nm}-OD_{630 nm}<0.1

Normal range of Positive control (1:1600): OD_{450 nm}-OD_{630 nm}>1.0

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 \geq Cut-off value means Anti-Influenza A (Wisconsin/67/2022) HA

Antibody IgG are detected.

Negative reading: $OD_{450\text{ nm}} - OD_{630\text{ nm}}$ of sample < Cut-off value means Anti-Influenza A (Wisconsin/67/2022) 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 solution is 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} =0.059	Negative	Anti-Influenza A (Wisconsin/67/2022) HA Antibody IgG are not detected
OD _{450 nm} - OD _{630 nm} =0.427	Positive	Anti-Influenza A (Wisconsin/67/2022) HA 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
400	3.246	The titer level of antibody is 51200
800	3.047	
1600	2.487	
3200	1.582	
6400	0.982	
12800	0.487	
25600	0.245	
51200	0.135	
102400	0.081	
Blank	0.019	