RA189-EN.01

Influenza A (H1N1) Viruses Hemagglutinin (HA) Specific ELISA Kit

Pack Size: 96 tests

Catalog Number：RAS-A189

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 Influenza A [A/Wisconsin/588/2019 (H1N1)] viruses Hemagglutinin (HA), Influenza A [A/Victoria/4897/2022 (H1N1)] viruses Hemagglutinin (HA) and Influenza A [A/Wisconsin/67/2022 (H1N1)] viruses Hemagglutinin (HA) in samples. 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 (Influenzavirus A, Influenzavirus B, and Influenzavirus 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 levels of Influenza A (H1N1) viruses Hemagglutinin (HA) by employing a standard sandwich-ELISA format. The microplate in the kit has been pre-coated with Anti-HA (Influenza A (H1N1)) Antibody. First add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the HRP-HA (Influenza A (H1N1)) 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 Influenza A (H1N1) viruses Hemagglutinin (HA) present. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450nm and 630nm. The OD Value reflects the amount of Influenza A (H1N1) viruses Hemagglutinin (HA) bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS189-C01	Pre-coated Anti-HA (Influenza A (H1N1)) Antibody Microplate	1 plate	Solid	2-8°C	2-8°C

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RAS189-C02	HA (Influenza A/Wisconsin/588/2019 (H1N1)) Standard	20 μg	Powder	2-8℃	-70℃
RAS189-C03	HRP-HA (Influenza A (H1N1)) Antibody	20 μg	Powder	2-8℃, avoid light	-70℃, avoid light
RAS189-C04	10×Washing Buffer	50 mL	Liquid	2-8℃	2-8℃
RAS189-C05	2×Dilution Buffer	50 mL	Liquid	2-8℃	2-8℃
RAS189-C06	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
RAS189-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 components table. 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

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

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

ID	Components	Size	Stock Solution Con.	Reconstitution Buffer and
RAS189-C02	HA (Influenza A/Wisconsin/588/2019 (H1N1)) Standard	20 μg	200 μg/mL	100 μL water
RAS189-C03	HRP-HA (Influenza A (H1N1)) 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-HA (Influenza A (H1N1)) Antibody working fluid:

Dilute HRP-HA (Influenza A (H1N1)) Antibody to 1.0 μg/mL with 1×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 HA (Influenza A/Wisconsin/588/2019 (H1N1)) as a Standard curve with Dilution Buffer as recommended in Figure 1.

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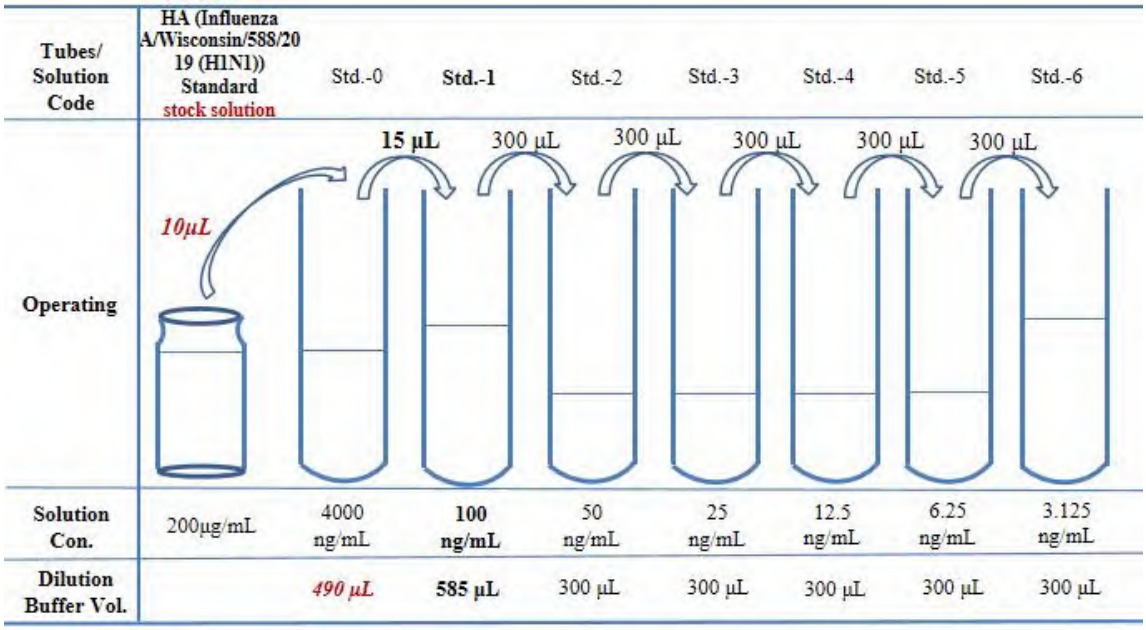
FIGURE 1. PREPARATION OF 1:1 SERIAL DILUTIONS OF THE HA (Influenza A/Wisconsin/588/2019 (H1N1))



3. Add Samples

Add 50 μL serially diluted **HA (Influenza A/Wisconsin/588/2019 (H1N1))** Standard curve and samples to each well. For blank Control wells, please add 100μL 1×Dilution Buffer. Then add 50 μL **HRP-HA (Influenza A (H1N1)) Antibody (dilute to 1.0 μg/mL)** working fluid to each well. Shake gently to mix. Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

4. Washing

Remove the remaining solution by aspiration, add 300 μL of 1×Washing Buffer to each well, soak for 30s, 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. 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.

6. Termination

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

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

7. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 5 minutes.

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: 1.563-50 ng/mL.

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

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

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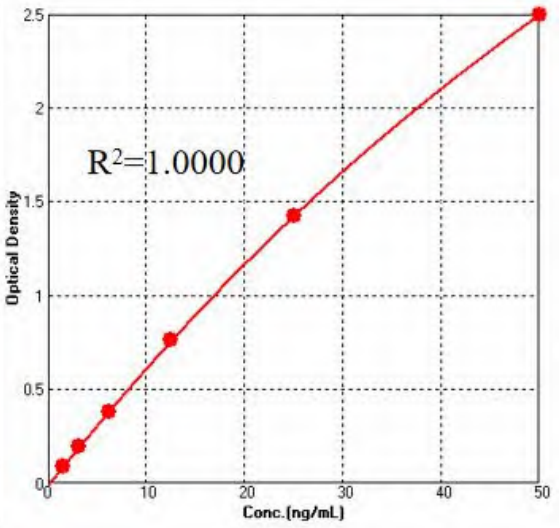
Standard (ng/mL)	O.D.-1	O.D.-2	Average	Corrected
50	2.516	2.522	2.519	2.497
25	1.489	1.401	1.445	1.423
12.5	0.791	0.775	0.783	0.761
6.25	0.402	0.401	0.402	0.379
3.125	0.219	0.216	0.218	0.195
1.563	0.109	0.112	0.111	0.088
0	0.023	0.023	0.023	/



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)	36.153	9.071	4.069	36.228	9.011	4.162
SD	1.231	0.409	0.257	0.132	0.081	0.159
CV (%)	3.4	4.5	6.3	0.4	0.9	3.8

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

RECOVERY

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

Sample(n=5)	Average Recovery %	Range %
High	103.5	97.9-109.6
Middle	102.9	95.2-111.3

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Low	99.5	94.2-105.3

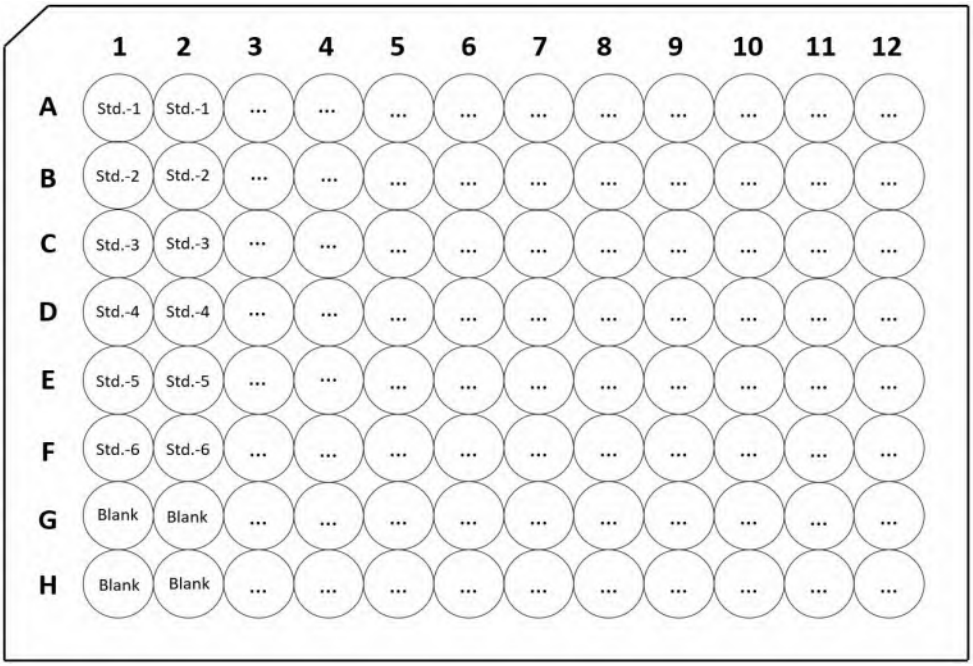
LINEARITY

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 (1640)
1:2	Average Recovery (%)	89.1	87.7
	Range (%)	84.1-91.8	83.6-90.0
1:4	Average Recovery (%)	88.2	86.1
	Range (%)	85.9-90.4	81.6-92.4
1:8	Average Recovery (%)	95.7	93.5
	Range (%)	93.4-99.0	83.3-98.3
1:16	Average Recovery (%)	96.7	97.1
	Range (%)	91.0-103.8	89.6-100.6

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

PLATE LAYOUT



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Note: *Blank is a Blank Dilution Buffer hole.*

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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