Swine H1N1 HA ELISA Kit

Catalog Number KA1292

96 assays

Version: 1.1

Intended for research use only

www.abnova.com

Table of Contents

Introduction 3

Background 3

Principle of the Assay 3

General Information 4

Materials Supplied 4

Storage Instruction 4

Materials Required but Not Supplied 4

Precautions for Use 5

Assay Protocol 6

Reagent Preparation 6

Assay Procedure 7

Data Analysis 8

Calculation of Results 8

Performance Characteristics 8

Resources 10

References 10

Plate Layout 11

Introduction

Background

H1N1 is a subtype of influenza virus A and the most common cause of influenza in humans. A swine-origin H1N1 strain cause the “2009 flu pandemic” in 2009, it is also referred to as "swine flu". The virus appeared to be a new strain of H1N1 which resulted when a previous triple reassortment of bird, swine and human flu viruses further combined with a Eurasian pig flu virus[1]. Most people have no or little immunity to this new virus strain, this cause more infections than are seen with seasonal flu. The virus spread worldwide by human-to-human transmission, causing the World Health Organization to raise its pandemic alert to the highest level 6 in 2009.

Hemagglutinin (HA) is a single-pass type I integral membrane glycoprotein from the influenza virus, and comprises over 80% of the envelope proteins present in the virus particle. It has two functions. Firstly, it allows the recognition of target vertebrate cells, accomplished through the binding of these cells' sialic acid-containing receptors. Secondly, once bound it facilitates the entry of the viral genome into the target cells by causing the fusion of host endosomal membrane with the viral membrane[2]. HA plays a major role in the determination of host range restriction and virulence.

Principle of the Assay

The design of this assay is based on a sandwich Enzyme-Linked Immunosorbent Assay (ELISA) for the quantitative determination of swine H1N1 (A/California/04/2009) HA. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to Swine H1N1 (A/California/04/2009) HA. Samples and the standard protein are pipetted into these wells. Unbound components in the sample are removed by washing, and then biotin-conjugated monoclonal antibody specific to H1N1 (A/California/04/2009) HA is added, then washed again, and peroxidase-conjugated streptavidin is added, producing an antibody-antigen-antibody “sandwich”. The final step, an OPD-substrate solution is added to each well for the color development. After appropriate time of incubation, a sulfuric acid solution is added to stop the color developing reaction, and the resulting yellow colored product is measured at 490 nm with a microtiter plate reader. The intensity of yellow color is proportional to the H1N1 (A/California/04/2009) HA amount of sample captured in plate.

General Information

Materials Supplied

List of component

Component	Amount
Swine H1N1 Antibody Coated Stripwell Microplate	96-wells (8-well Strips x12)
Detection Antibody	10 µL/vial x1
Streptavidin-HRP	5 µL/vial x1
Protein Standard Stock	2 µg/vial x1
TMB Substrate Solution	11 mL/bottle x1
Diluent	40 mL/bottle x1
10x Solution A	30 mL/bottle x1
10x Solution B	50 mL/bottle x1
Adhesive plastic	3 pcs
Stop Solution	10 mL/bottle x1

1. Swine H1N1 Antibody Coated Stripwell Microplate: Anti-swine H1N1 microtiter plate, one plate of 96 wells (8-well strips x 12), coated with a mouse monoclonal antibody specific to swine H1N1 (A/California/04/2009) HA.
2. Detection Antibody: Biotin labeled mouse anti-swine H1N1 (A/California/04/2009) HA antibody
3. Streptavidin-HRP: Peroxidase-conjugated Streptavidin (HRP, enzyme)
4. Protein Standard Stock: Recombinant swine H1N1 (A/California/04/2009) HA protein.

- ✓ Do not mix or interchange different reagents from various kit lots
- ✓ Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine(TMB). Wear gloves and eye and clothing protection when handling these reagents. These solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary.

Storage Instruction

Store the Detection Antibody and Protein Standard Stock at -80℃, and aliquot to minimize the repeat freeze-thaw cycle. Store other components at 3~5℃. TMB should be keep away from light.

Materials Required but Not Supplied

- ✓ Microtiter plate reader capable of measurement at or near 450nm.
- ✓ Calibrated, adjustable precision pipettes, preferably with disposable plastic tips (A manifold multi-channel

pipette is desirable for large assays.)

- ✓ Distilled or deionized water
- ✓ Data analysis and graphing software
- ✓ Vortex mixer
- ✓ Polypropylene tubes for diluting and aliquoting standard
- ✓ Absorbent paper towels
- ✓ Calibrated beakers and graduated cylinders of various sizes
- ✓ Micro-plate Shaker

Precautions for Use

- ✓ This kit has been configured for research use only and is not to be used in diagnostic and clinical use.
- ✓ Wear gloves and laboratory coats when handling.
- ✓ The materials must not be pipetted by mouth.
- ✓ Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.

Assay Protocol

Reagent Preparation

- Protein Standard

Prepare 1 mL of 100 ng/mL swine H1N1 standard from Protein Standard Stock by diluting with the Diluent, and create the following serial dilutions from it by further dilute with Diluent :

Standard Point	Concentration	Preparation Note
P1	10 ng/mL	Take 0.1 mL of 100 ng/mL Protein Standard and dilute with 0.9 mL Diluent
P2	3.33 ng/mL	Take 200 µL solution of P1 and dilute with 400 µL Diluent
P3	1.11 ng/mL	Take 200 µL solution of P2 and dilute with 400 µL Diluent
P4	0.37 ng/mL	Take 200 µL solution of P3 and dilute with 400 µL Diluent
P5	0.12 ng/mL	Take 200 µL solution of P4 and dilute with 400 µL Diluent
P6	0 ng/mL	400 µL of the Diluent

- Detection Antibody

1. Mix gently before use.
2. Add 1 µL Detection Antibody into 5 mL of Diluent, Mix gently. Make further 10 fold dilution by dilute with Diluent and mix gently. Label as “Working Detection Antibody Solution”.
3. Return the unused Detection Antibody to the refrigerator.

- 1X Solution A

1. Equilibrate to room temperature, mix to re-dissolve any precipitated salt.
2. Mix 1 volume 10X Solution A + 9 volumes of deionized water. Label as “Working Solution A”.
3. Store both the concentrated and the Working Solution A in the refrigerator.

- 1X Solution B (Wash buffer)

1. Equilibrate to room temperature, mix to re-dissolve any precipitated salt.
2. Mix 1 volume 10X Solution B + 9 volumes of deionized water. Label as “Working Solution B”.
3. Store both the concentrated and the Working Solution B in the refrigerator.

- Streptavidin-HRP

1. Mix gently before use.
2. Add 1µL Streptavidin-HRP to 8 mL 1x Solution A, mix gently. Make additional 10 fold dilution by dilute with 1x Solution A and mix gently. Label as “Working STA-HRP Solution”.
3. Return the unused Streptavidin-HRP to the refrigerator.

- TMB Substrate Solution

Equilibrate to room temperature before use.

- Stop solution

Equilibrate to room temperature before use.

Assay Procedure

1. Rehydrate the plate: Apply 100 µL of Working Solution A in the wells; incubate 10 minutes at room temperature (RT).
2. Wash the plate one time with Working Solution B. The solutions are removed by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel.
3. Add 100 µL of Protein Standard (P1 ~ P6) and samples into respective well. Cover the plate with an Adhesive plastic and incubate for 2 hrs at RT with shaking at 80 rpm on an orbital microplate shaker.
4. Wash the plate six times with Working Solution B.
5. Apply 100 µL of Working Detection Antibody Solution. Cover the plate with an Adhesive plastic and incubate for 2 hrs at RT with shaking at 80 rpm on an orbital microplate shaker.
6. Wash the plate eight times with Working Solution B.
7. Apply 80 µL Working STA-HRP Solution. Cover the plate with an Adhesive plastic and incubate for 1 hr at RT with shaking at 80 rpm on an orbital microplate shaker.
8. Wash the plate ten times with Working Solution B.
9. Apply 100 µL TMB Substrate Solution into each well. Incubate in dark environment, e.g. cover the plate with aluminum foil. Do not shake the plate during the incubation.
10. Monitor the absorbance at 650 nm (OD650) in a microplate reader regularly, e.g. every 2 minutes, until the OD650 value of Protein Standard P1 reach approximately 1.0, then add Stop Solution (100 µL/well) to stop the color development.
11. Read the absorbance at 450 nm (OD450) with an ELISA plate reader.

- Directions for washing

✓ Fill the wells with 200 µL of “Working Solution B”. Let it soak for 1 to 3 minutes and then all residual wash-liquid must be drained from the wells by aspiration (beware not to scratch the inside of the well) or decantation, followed by forceful tapping of the plate on absorbent paper. Never insert absorbent paper directly into the wells. If using an automated washer, the operating instructions for washing equipment should be carefully followed.

✓ Incomplete washing will adversary affects the assay and renders false results.

✓ It is recommended to use laboratory tape to hold plate strips to the plate frame while performing the plate washing to avoid strips coming free of the frame.

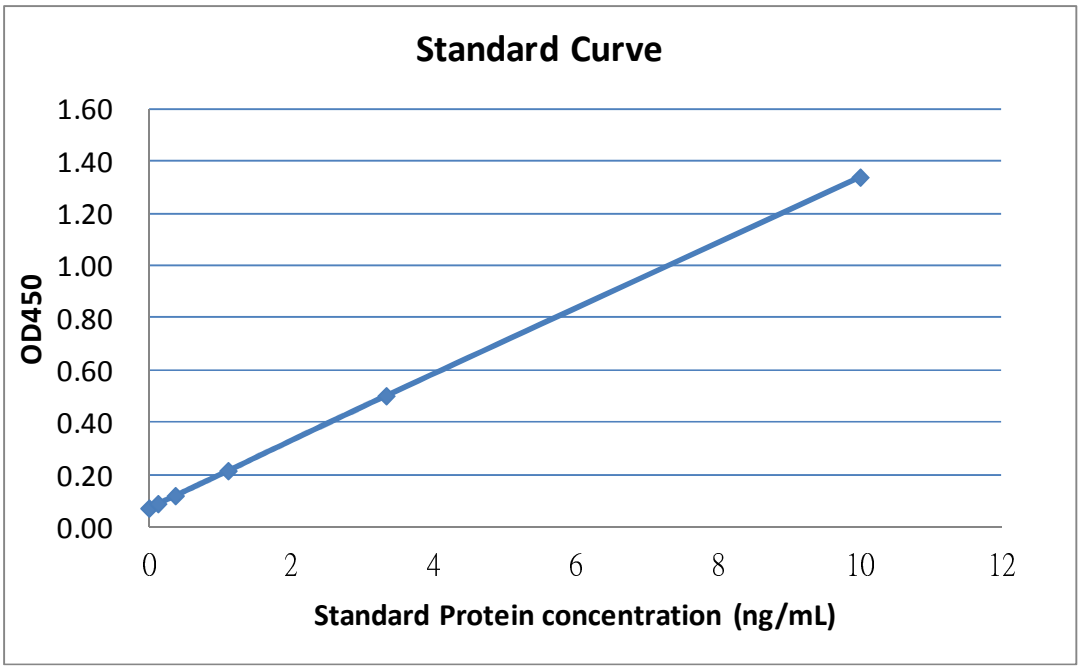
Data Analysis

Calculation of Results

The standard curve below is for illustration only and should not be used to calculate results in your assay.

A standard curve must be run with each assay.

Concentration of Protein Standard (ng/mL)	OD450
10	1.339
3.33	0.503
1.11	0.2165
0.37	0.1215
0.12	0.0905
0	0.0735



Performance Characteristics

- Sensitivity

The minimal detectable dose of Swine H1N1 was calculated to be 0.12 ng/mL.

- Specificity

This ELISA Kit shows no detectable cross-reactivity with H5N1.

- Precision

1. Intra-Assay (Within-Run, n=2)

Protein Conc. (ng/ml)	OD450nm Mean	SD	CV (%)
0	0.074	0.009	12.51%
0.12	0.091	0.008	8.59%
0.37	0.122	0.004	2.91%
1.11	0.217	0.012	5.55%
3.33	0.503	0.006	1.12%
10	1.339	0.020	1.48%

2. Inter-assay (Run-to-Run, n=2)

Protein Conc. (ng/ml)	OD450nm Mean	SD	CV (%)
0	0.073	0.001	0.97%
0.12	0.093	0.003	3.06%
0.37	0.124	0.003	2.57%
1.11	0.224	0.010	4.43%
3.33	0.527	0.033	6.31%
10	1.414	0.106	7.52%

Resources

References

1. Trifonov V, Khiabanian H, Rabadan R. (2009). Geographic Dependence, Surveillance, and Origins of the 2009 Influenza A (H1N1) Virus. N Engl J Med. 2009 Jul 9;361(2):115-9. Epub 2009 May 27.
2. White JM, Hoffman LR, Arevalo JH, et al. (1997). Attachment and entry of influenza virus into host cells. Pivotal roles of hemagglutinin. In Chiu W, Burnett RM, Garcea RL. Structural Biology of Viruses. Oxford University Press. pp. 80–104.

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H