PrioCHECK™ Swine Influenza Ab Serum Plate Kit

Immunoenzymatic test for specific detection of anti-influenza antibodies in swine serum

Catalog Number VETSIV5I **Doc. Part No.** 100020286 **Pub. No.** MAN0008640 **Rev.** B.0

Technology	Species	Sample matrix	Test type	Protocol
Indirect ELISA – Strip plates	Swine	Serum	Individual	Short incubation

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

WARNING! POTENTIAL BIOHAZARD. Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

General information

Swine flu is a highly contagious swine (porcine) viral infection. The Swine Influenza Virus (SIV) causes a respiratory disease characterized by coughing, sneezing, nasal discharge, high rectal temperature, lethargy, labored breathing and a drop in appetite. In certain cases, SIV infections are associated with reproduction difficulties such as abortion.

The Applied Biosystems™ PrioCHECK™ Swine Influenza Ab Serum Plate Kit is a fast and specific method to detect anti-influenza virus antibodies in swine serum. This kit is capable of detecting antibodies against type A virus of the Swine Flu (H1N1 and H3N2).

Procedure overview

The test is based on the principle of an **indirect ELISA assay.**

1. Samples and controls are distributed to the Influenza (Flu) viral antigen-coated plate. Any specific anti-Flu antibodies that are present bind to the antigen.
2. After washing, an anti-pig IgG conjugate labeled with peroxidase is added and binds to the antibodies previously attached to the microwells.
3. The unbound conjugate is eliminated by washing, followed by the addition of a chromogenic substrate. A green color results from substrate oxidation by the peroxidase in the conjugate.
4. After stopping the reaction, the color remains green. The results are read by an ELISA plate reader.

The appearance of a green color indicates a positive sample. The color of each well is proportional to the level of specific antibodies present in the sample.

Kit reagents and storage

Component	Description	Quantity (480 tests)	Storage
1 - Coated microplate Flu	Influenza antigen-coated plate, 12 strips of 8 wells	5 units	5±3°C[1]
2 - Negative C. Flu	Negative Control, Influenza (NC, blue)	2 mL	5±3°C
3 - Positive C. Flu	Positive Control, Influenza (PC, yellow)	2 mL	
4 - Conjugate Flu	Conjugate Influenza, anti-pig HRP (pink)	30 mL	
A - Wash (10x)	Wash solution, 10X concentrate	250 mL	
B - Sample DB (3x) Flu	Sample diluent buffer Influenza, 3X concentrate (green)	100 mL	
C - Substrate	Substrate solution (ABTS)	30 mL	
D - Stop	Stop solution (oxalic acid)	30 mL	
Adhesive plate covers		10	RT[2]

[1] Unused strips can be stored in the sealed pouch with desiccant (supplied with the kit) at 5±3°C until the kit's expiration date.
[2] Room temperature

Materials required but not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Single- and multi-channel micropipettes	Disposable pipette tips
Distilled or deionized water	Microplate incubator (37±2°C)
Pre-dilution plates	ELISA reader equipped with a 405 nm filter
Disposable containers	—

Important procedural guidelines

- Do not mix reagents from different kit batches.
- Avoid contaminating the reagents by using single-use sampling equipment.
- Do not pipette reagents by mouth.

Sample information

Fresh, refrigerated sera (8 days at 5±3°C) or frozen sera (1 year at < −16°C) can be used.
The samples are thoroughly mixed and tested at a **1:200** dilution.

Prepare reagents

- The reagents **1 - Coated microplate Flu, 2 - Negative C. Flu, 3 - Positive C. Flu, 4 - Conjugate Flu, C - Substrate,** and **D - Stop** are ready for use.
- The **B - Sample DB (3x) Flu** reagent must be diluted **1:3 in distilled/deionized water**.
  Example: dilute 20 mL of **B - Sample DB (3x) Flu** reagent in 40 mL of water.
  Mix after diluting. The **diluted Sample DB Flu** solution can be stored at 5±3°C and used within 2 days.
- **A - Wash (10x)** solution must be diluted **1:10 in distilled/deionized water.**
  Example: for one strip: 2 mL of **A - Wash (10x)** solution in 18 mL of water; for one plate: 25 mL of **A - Wash (10x)** solution in 225 mL of water.
  Mix after diluting. The **diluted Wash** solution can be stored for 1 month at 5±3°C.

NOTE: Due to the high salt concentration, crystals may form in the **A - Wash (10x)** and **B - Sample DB (3x) Flu** solutions. Prior to dilution, shake the bottle to dissolve any crystals.

Perform the ELISA test

NOTE: Bring the reagents to room temperature (21±4°C) before performing the test. The tolerance range for incubation times is ±10%. The use of disposable containers is recommended for distribution of components.

1. Distribution of samples and controls

A. **In a pre-dilution plate, pre-dilute the sera to be analyzed 1:20 in diluted Sample DB Flu** (see "Prepare reagents"):
   1. Add **5 µL of each serum** to the pre-dilution plate. Keep the same order that will be used on the coated plate.
   2. Then add **95 µL of diluted Sample DB Flu** to each sample well. Gently shake the plate.
   3. Let the diluted sera equilibrate in the diluted Sample DB Flu at room temperature for 5 minutes before transferring to the coated plate.

   NOTE: The pre-diluted sera can be stored for 24 hours at 5±3°C.

B. **To obtain the final 1:200 dilution of the sera, perform a second 1:10 dilution in diluted Sample DB Flu in the coated plate:**
   - Remove the adhesive plate cover from the coated plate.
   - Add **5 µL of serum sample, pre-diluted 1:20,** to the appropriate sample wells.
   - Add **45 µL of diluted Sample DB Flu** to each sample well.

C. Add **50 µL of each control** to be analyzed **undiluted**:
   - Add **50 µL** of **2 - Negative C. Flu** reagent to wells A1 and B1 (for example).
   - Add **50 µL** of **3 - Positive C. Flu** reagent to wells C1 and D1 (for example).

Gently shake and cover the plate, using a new adhesive plate cover. **Incubate the plate for 1 hour at 37±2°C.**

2. Washing steps

Empty the plate and **perform 3 washes** with the **diluted Wash** solution (see "Prepare reagents") using **300 µL** per well. Empty and tap the plate on absorbent paper to remove all traces of liquid. Washes can be performed either **manually** or using an **automated plate washer**. Do not allow the plate to dry out.

3. Distribution of conjugate

Add **50 µL** of **4 - Conjugate Flu** reagent to each well. Gently shake and cover the plate, using a new adhesive plate cover. **Incubate the plate for 1 hour at 37±2°C.**

4. Washing steps

Repeat the **Washing steps** as described above.

5. Test development

Add **50 µL** of **C - Substrate** solution to each well. Gently shake the plate for 2 seconds. **Incubate for 15 minutes at room temperature (21±4°C) in darkness.** Do not cover the plate.
Add **50 µL of D - Stop** solution to each well and in the same order as the **C - Substrate** solution. Gently shake the plate for 2 seconds.

6. Reading

Wipe the bottom of the plates with a soft tissue to remove any dust. Read the plate within 30 minutes after stopping the reaction, at **405 nm**.

Calculation

Calculate the average OD of the PC ($OD_{m\,PC}$), and that of the NC ($OD_{m\,NC}$).
For each sample, calculate the IRPC value (sample value divided by the positive value × 100):

$$IRPC = [(OD_{Sample} - OD_{m\,NC}) / (OD_{m\,PC} - OD_{m\,NC})] \times 100$$

NOTE: For negative samples, an IRPC value **<0** is possible.

Validation

The test is validated if:

$$OD_{m\,PC} > 0.700 \text{ and } (OD_{m\,PC} / OD_{m\,NC}) > 5.5$$

Interpretation of results

Results	Interpretation
IRPC ⩽ 20	Negative
IRPC > 20	Positive

Quantitative interpretation

Results	Interpretation
< 20	Negative
20 ⩽ Titer ⩽ 60	Positive: Insufficient vaccination protection
60 < Titer ⩽ 100	Positive: Good vaccination protection
> 100	Positive: Suspected infection, whether or not the animals are vaccinated

Immunological profile interpretation following vaccination

To interpret the results of the PrioCHECK™ Swine Influenza Ab Serum Plate Kit, it is necessary to analyze 10 to 20 animals from the same group receiving the same vaccine protocol.

It is also necessary to perform an immunological profile with a calculation of the Average Titer and the CV (%).

CV (%)	Average Titer of the group			
	< 20	20 ⩽ Titer ⩽ 60	60 < Titer ⩽ 100	> 100
0 to 30%	No protection	Insufficient protection	Homogeneous vaccination	Group suspected of infection
30 to 50%			Inhomogeneous vaccination	
> 50%			Heterogeneous vaccination	

Documentation and support

Customer and technical support

Technical support: visit **thermofisher.com/askaquestion**

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Order and web support
- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)

  NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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A.0	24 February 2014	Baseline for revision history