PrioCHECK™ African Swine Fever Virus Ab Kit

ELISA for *in vitro* detection of antibodies against African swine fever virus in serum of pigs and wild boars

Catalog Number A56981

Pub. No. MAN0019591 Rev. A.0

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.

Technology	Species	Sample matrix	Test type
Indirect ELISA	Porcine and wild boar	Serum	Individual

Product description

The Applied Biosystems™ PrioCHECK™ African Swine Fever Virus Ab Kit (Cat. No. A56981) is an easy-to-use ELISA that enables detection of antibodies directed against the p30 protein of African swine fever virus (ASFV) in porcine serum. The assay meets the sensitivity and specificity recommendations of the World Organisation for Animal Health (OIE) to detect ASFV infection and is compatible with automated plate washers.

ASFV is a double-stranded DNA virus of the family *Asfarviridae*. African swine fever, caused by ASFV, is a highly contagious, lethal disease in pigs and wild boars that causes high fever, skin and internal organ hemorrhages, and loss of appetite. ASFV spreads rapidly in pig populations through direct or indirect contact, and through the bite of infected *Ornithodoros moubata* soft tick species. Outbreaks of ASFV cause severe economic losses in pig-producing countries, making rapid detection of infected pigs essential to control the disease. Given the long-term persistence of anti-ASFV antibodies after infection, serology tests provide an accurate and easy approach to control the health status of porcine herds and wildlife (wild boars).

Contents and storage

Reagents and plates for 460 tests are supplied.

Component	Amount	Storage [1]
1: Test Plate	5 × 12-strip plates[2]	2–8°C
2: Positive Control (PC)	600 µL	
3: Negative Control (NC)	600 µL	
4: ASFV Sample Dilution Buffer	150 mL	
5: ASFV Conjugate	60 mL	
6: Chromogen (TMB) Substrate	60 mL	
7: Stop Solution	60 mL	
8: Washing Fluid (20X)	2 × 60 mL[3]	
Plate sealers	10	

[1] See the label for the expiration date.

[2] Twelve 8-well strips (coated with ASFV recombinant p30 antigen) in a plate frame; sealed in a plastic bag with a desiccant sachet.

[3] Sufficient to prepare 2.4 L of wash solution (1X).

Required materials not supplied

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

Item	Source[1]
Multiskan™ FC Microplate Photometer, or equivalent[2]	51119000
Single channel pipette (2–20 µL)	MLS
Multichannel pipette (50–500 µL)	MLS
Pipette tips (as recommended by pipette manufacturer)	thermofisher.com/pipettetips
Laboratory mixer (vortex or equivalent)	MLS
Solution reservoirs	MLS
Additional plate (for pre-dilution)	MLS
Deionized or distilled water	MLS
(*Optional*) Microplate shaker	MLS
(*Optional*) Wellwash™ Microplate Washer, or equivalent	5165000

[1] "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier.

[2] Plate reader must be capable of measurement at 450 nm.

For Veterinary Use Only. For In Vitro Use Only.

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Workflow

Note: The following times are based on the estimated processing time for one Test Plate.

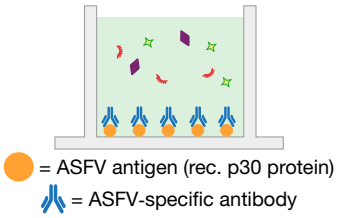
PrioCHECK™ African Swine Fever Virus Ab Kit workflow

65 min

Incubate samples and controls

1. Add ASFV Sample Dilution Buffer to all wells of the pre-dilution plate.
2. Add samples and controls to the pre-dilution plate containing ASFV Sample Dilution Buffer (1:50 final dilution), then mix.
3. Transfer the sample dilution to the Test Plate (pre-coated with ASFV antigen).
4. Incubate at room temperature for 60 minutes.

ASFV-specific antibodies bind to the antigen, forming antigen-antibody complexes.

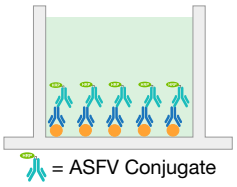


35 min

Add ASFV Conjugate

Wash the Test Plate, then incubate the samples with HRP-labeled ASFV Conjugate for 30 minutes.

The ASFV Conjugate binds to ASFV-specific antibodies if present in the sample.

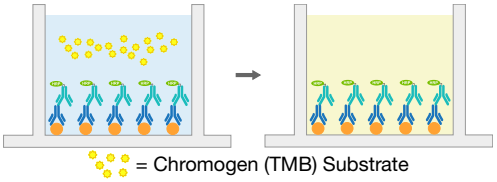


20 min

Perform the substrate reaction, then read the plate

1. Wash the Test Plate, then incubate the samples with Chromogen (TMB) Substrate for 15 minutes.
2. Add Stop Solution to the wells, then read the plate.

Color development indicates the presence of ASFV-specific antibodies in the sample.



5 min

Analyze the results

1. Ensure the validation criteria are met.
2. Calculate the percent positivity for each sample, then interpret the results.

Procedural guidelines

- Strictly follow all national safety regulations.
- Perform the kit protocol in laboratories that are suited for its purpose.
- Consider samples as potentially infectious and all items that contact the samples as potentially contaminated.
- Strictly follow the procedure described in this user guide.

  Note: Incubation times can vary ±10% without affecting assay performance.
- All kit components are ready for use, with the exception of Washing Fluid (20X).
- Throughout this user guide room temperature is defined as 21 ± 3°C.
- Equilibrate all kit components to room temperature before use.
- Change pipette tips after every pipetting step.
- Maintain separate solution reservoirs for each reagent with the needed volume to perform the assay. Do not return unused reagents to the bottle.
- Do not use kit components after their expiry date or if you observe changes in their appearance.
- Do not mix kit components from different kit lot numbers.
- Use deionized or distilled water, or water of equivalent quality.
- Use serum samples that are fresh, refrigerated (<8 days at 2–8°C), or frozen (<1 year at –25°C to –15°C).
- *(Recommended)* Use a microplate washer for all washing steps. Insufficient washing can result in high background.

Before you begin

Prepare the reagents and Test Plate

- Equilibrate all kit components to room temperature, then gently mix reagents, samples, and controls.
- Determine the number of 8-well strips that are required for the assay, then place the strips in the plate frame.

  Seal any unused strips in a plastic bag with the provided desiccant sachet, then store at 2–8°C for future use.

Prepare wash solution (1X)

1. If Washing Fluid (20X) shows a precipitate, shake the bottle until the precipitate is dissolved. Alternatively, warm the Washing Fluid (20X) at 30–40°C with magnetic stirring to accelerate the dissolution.
2. Prepare wash solution (1X)—Combine 1 part Washing Fluid (20X) with 19 parts deionized or distilled water, then mix the solution thoroughly.

   Example: For one Test Plate, add 12.5 mL of Washing Fluid (20X) to 237.5 mL of deionized or distilled water.

   Note: If a plate washer is used, the volume should be adjusted to include the volume needed to prime the plate washer.

Store the wash solution (1X) at room temperature for up to one week, or at 2–8°C for up to one month.

Perform the ELISA procedure

1 Incubate samples and controls

1. Dilute samples and controls with ASFV Sample Dilution Buffer in the pre-dilution plate, according to the following table.

   Depending on the precision pipettes available, the dilution can be performed in one step using 5 µL of sample or two steps using 10 µL or 20 µL of sample. The one-step dilution and the first step of the two-step dilution are performed in the pre-dilution plate.

   The sample and control final dilutions are a 1:50 ratio.

Component	One-step dilution	Two-step dilution		Well position[1]
	Volume for 5-µL sample	Volume for 10-µL sample	Volume for 20-µL sample	
ASFV Sample Dilution Buffer	245 µL	90 µL	80 µL	All wells
Positive Control (PC)	5 µL	10 µL	20 µL	A1 and B1
Negative Control (NC)	5 µL	10 µL	20 µL	C1 and D1
Test sample	5 µL	10 µL	20 µL	One well (single test), or two adjacent wells (duplicate test)

[1] Suggested position in the pre-dilution plate.

2. After all samples and controls are dispensed, mix well by pipetting up and down at least 5 times.
3. Transfer each sample and control dilution from the pre-dilution plate to the corresponding wells in the Test Plate.

   If you are performing a two-step dilution, add ASFV Sample Dilution Buffer first to the Test Plate.

Component	One-step dilution	Two-step dilution		Well position[1]
	Volume to transfer for 5-µL sample	Volume to transfer for 10-µL sample	Volume to transfer for 20-µL sample	
ASFV Sample Dilution Buffer	—	80 µL	90 µL	All wells
Sample or control dilution from the pre-dilution plate	100 µL	20 µL	10 µL	Corresponding well(s) in the Test Plate

[1] Suggested position in the Test Plate.

4. If the two-step dilution was performed, mix the contents of the wells thoroughly. Use one of the following methods.
   - Using a plate shaker—Cover the plate with a plate sealer, then shake the plate at 600 rpm for 1 minute.
   - By carefully pipetting—Pipet up and down at least 5 times, then cover the plate with a plate sealer.
5. If the one-step dilution was performed, cover the plate with a plate sealer.
6. Incubate the plate for 60 minutes at room temperature.

2 Add ASFV Conjugate

1. Empty the Test Plate, then wash the wells 3 times with 350 µL/well of wash solution (1X) according to your washing method.

   IMPORTANT! Do not allow the plate to dry out.

   - **Manual washing—**Repeatedly fill, then empty the wells by aspiration or inversion. After the last wash cycle, tap the plate over a paper towel or absorbent surface to remove as much liquid as possible from the wells.
   - **Using an automated plate washer—**Wash the wells, following the guidelines for your instrument.
2. Add 100 µL of ASFV Conjugate to the Test Plate.
3. Cover the plate with a plate sealer.
4. (*Optional*) Gently shake the plate.
5. Incubate the plate for 30 minutes at room temperature.

3 Perform the substrate reaction, then read the plate

1. Empty the Test Plate, then wash the wells 3 times with 350 µL/well of wash solution (1X) according to your washing method.
   - **Manual washing—**Repeatedly fill, then empty the wells by aspiration or inversion. After the last wash cycle, tap the plate over a paper towel or absorbent surface to remove as much liquid as possible from the wells.
   - **Using an automated plate washer—**Wash the wells, following the guidelines for your instrument.
2. Add 100 µL of Chromogen (TMB) Substrate to the Test Plate.

   Note: Start the 15-minute incubation time (step 3.4) when Chromogen (TMB) Substrate is added to the first well.
3. (*Optional*) Gently shake the plate.
4. Incubate the Test Plate for 15 minutes at room temperature in the dark.

3	Perform the substrate reaction, then read the plate *(continued)*	5. Add 100 µL of Stop Solution to the Test Plate in the same order that Chromogen (TMB) Substrate was dispensed. 6. Gently shake the Test Plate. 7. Measure the optical density (OD) of the wells using a plate reader at 450 nm within 10 minutes after color development has stopped.

Analyze the results

Calculate the results

1. Calculate the mean OD_{450} value of the Positive Control (mean of wells A1 and B1 = POS OD_{450}).
2. Calculate the mean OD_{450} value of the Negative Control (mean of wells C1 and D1 = NEG OD_{450}).
3. Calculate the corrected OD_{450} of the Positive Control:

   Corrected POS $OD_{450\ PC}$ = POS OD_{450} – NEG OD_{450}
4. Calculate the corrected OD_{450} of the test samples:

   Corrected Sample OD_{450} = Sample OD_{450} – NEG OD_{450}
5. For each sample, calculate the percentage positivity (PP%):

   PP% = (corrected Sample OD_{450} / corrected POS OD_{450}) × 100
6. Calculate the coefficient of variation (CV%) for the Positive Control in OD_{450} of the duplicates (in wells A1 and B1).

   CV% = Standard Deviation (Positive Control OD_{450} duplicates) / (POS OD_{450})
7. Calculate the ratio POS OD_{450} / NEG OD_{450}.

Depending on the plate reader used, an OD_{450} >3.0 may be observed for some samples. This result can be considered positive, and samples do not need to be retested.

Validation criteria

If the following criteria are not met, the results are invalid and the samples must be retested.

- The coefficient of variation (CV%) between Positive Control OD_{450} duplicates must be <15%.
- The NEG OD_{450} must be ≤0.250.
- The POS OD_{450} must be ≥0.400.
- The ratio of POS OD_{450} / NEG OD_{450} should be ≥5.

Interpretation of results

Interpret the results according to the following table.

PP% result	Assay result	Interpretation
PP% ≥ 25	Positive	ASFV-specific antibodies are present in the test sample.
PP% < 25	Negative	ASFV-specific antibodies are not present in the test sample.

Note: PP% results greater than 100 are considered valid and indicate the presence of ASFV-specific antibodies in the test sample.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0019591

Revision	Date	Description
A.0	20 December 2022	New document created for PrioCHECK™ African Swine Fever Virus Ab Kit.