AffiVET® ASFV Detection Kit

SYMBOLOGY

Manufacturer
Caution
REF Catalog Number
−20 °C 30 °C Limits Temperature
Expiration Date
Consult Instructions for Use

1. DESCRIPTION

African swine fever is a serious viral disease of pigs, endemic in Africa. The African swine fever virus (ASFV), a large, enveloped DNA virus that replicates primarily in cells of the mononuclear phagocytic system, is highly contagious, and can spread very rapidly in pig populations by direct or indirect contact. This virus can persist for long periods in pig products and the environment. ASFV isolates vary in virulence from highly pathogenic strains that cause near 100% mortality to low–virulence isolates that can be difficult to diagnose.
AffiVET® ASFV Detection Kit is the direct detection of African swine fever virus on the basis of a genetic database, so it can diagnose very fast and accurately. It can amplify only specific gene using the PCR (Polymerase Chain Reaction) method, and take only 3 hours for detection. Therefore, it is a very fast, accurate and reliable technique.

2. STORAGE

The AffiVET® ASFV Detection Kit is shipped at room temperature (15–25°C) because contains a chemical stabilizer.
The AffiVET® ASFV Detection Kit should be stored immediately upon receipt at –20°C in a constant-temperature freezer. For routine use should be stored al 4°C. When stored under these conditions and handled correctly, these products can be kept at least until the expiration date without showing any reduction in performance.

3. KIT CONTENTS

KIT	48	96	
AffiVET® ASFV Premixture	1	1	vial
PCR Internal Control (white cap)	1	1	vial
DNase/RNase free water	1	1	vial
ASFV PCR Positive control	1	1	vial
PCR Negative control	1	1	vial
Mineral Oil Solution	1	1	vial
Molecular Weight marker	1	1	vial

4.MATERIALS

Materials required but not provided:
- Microcentrifuge and PCR tubes
- Disposable gloves, powderless
- Pipettes and tips
- DNA extraction kit
- Vortex mixer
- Centrifuge for microcentrifuge tubes
- Thermal cycler
- Tube racks
- Electrophoresis kit
- UV transilluminator
- Biohazard waste container

5.PROCEDURE

Please read through the entire procedure before starting.

5.1 DNA PREPARATION

This kit does not include DNA extraction kit. If you need a DNA extraction kit, please contact us at info@affigen.com.

Note: ⚠
Completely thaw the components of the kit prior to use; homogenize the solutions for several seconds prior to pipetting.

5.2 PREPARATION OF ASFV PCR MIXTURE

1) Prepare the reaction mixture for sample, positive control, negative control, and internal control by combining the reagents as shown in the table 1. The final reaction volume should be 13.5µl.

Notes:
- **Run a positive control, a negative control, and an internal control each 12 samples.**
- The mineral oil is necessary, even when using a thermal cycler that employs a top heating method.

Table 1. Reaction components for PCR

Kit components	Sample	Positive control	Negative control	Internal control
AffiVET® ASFV Premixture	5.5µL	5.5µL	5.5µL	
PCR Internal control (white cap)				5.5µL
DNase/RNase free water	6µl	6µl	6µl	6µl
DNA isolated from the sample	2µl			2µl
ASFV PCR Positive control		2µl		
PCR Negative control			2µl	
Mineral Oil Solution	11µl	11µl	11µl	11µl

2) Place the tubes in a thermal cycler and perform amplification according to the program outlined in Table 2.

Table 2. PCR cycling parameters

PCR cycle		Temp.	Time
1 cycle	Initial Denaturation	94ºC	2 min.
30 cycles	Denaturation	94ºC	30 sec.
	Annealing	56ºC	30 sec.
	Extension	72ºC	30 sec.
1 cycle	Final extension	72ºC	5 min.

5.3. DETECTION OF AMPLIFIED PRODUCTS

1) Prepare 1.5% agarose gel containing Ethidium bromide (Et-Br).
2) Load 7µl of PCR product, 7µl of positive control, 7µl of negative control, 7µl of internal control and 2µl of Brig™ Molecular Weight marker on agarose gel without adding a loading-dye buffer and perform electrophoresis.
3) Run electrophoresis by 100V (required about 30~40 minutes).
4) Identify the result on ultra-violet (UV) transilluminator.

5.4. INTERPRETATION OF THE TEST RESULTS

- Expected PCR product size : 277 bp

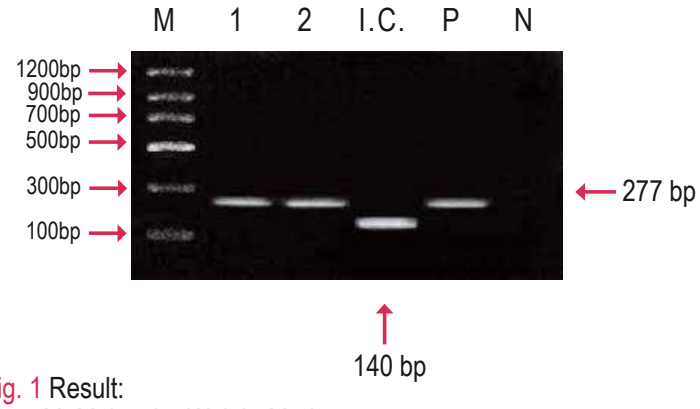

Fig. 1 Result:
Lane M: Molecular Weight Marker
Lane 1~2: ASFV Positive samples
Lane I.C.: Internal control
Lane P: Positive control
Lane N: Negative control

6. TROUBLESHOOTING

1) No band in positive sample

• Check Internal control band: If internal control band is seen, PCR has been performed properly. It is not a problem of the product.
• Check template DNA quality: the PCR reaction can be inhibited depending on DNA purity in some cases. In this case, extracted DNA should be diluted 10 times with DNA rehydration solution and used to perform PCR again.
• Check PCR machine: check the temperature and make sure to check that the machine is working properly.

2) No internal control band

• Check template DNA concentration: Competition can occur by high template concentration. Proceed with a lower concentration of DNA.
• Check template DNA quality: Even tough DNA is isolated from the sample, the PCR reaction can be inhibited depending on DNA purity in some cases. In this case, extracted DNA should be diluted 10 times with distilled water and used to run the PCR reaction again. If still no band is seen, please inquire with our technical support staff.

3) Amplicon bands in the negative control

• Check contamination of distilled water: Distilled water can be contaminated. Perform PCR again with fresh sterile water.
• Check contamination of laboratory instruments and other environments: We recommend that you use filter tips and a pipette after sterilization to reduce contamination. Proceed with all procedures on a clean bench and keep the location where you procedures are performed sterile.

4) Poor resolution on agarose gel

• We recommend using a 1.5~2% agarose gel and run electrophoresis for 40 minutes at 100 V.

7. TECHNICAL ASSISTANCE

If you experience any problems and/or issues while working with our products, please fo not hesitate to contact us at info@affigen.com

8. SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS).

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