MBPCR256 Hi-PCR® African Swine Fever Virus (ASFV) Probe PCR Kit

Description

African swine fever virus (ASFV) is the causative agent of ASF, the only member of the family Asfarviridae. It's a large double-stranded DNA virus, with a linear genome of 173–193 kb long, encoding 151–167 open reading frames (ORFs). It causes a highly contagious swine viral disease characterized by a wide range of clinical signs, from subclinical signs to sudden death that often occurs within 7 − 10 days. ASF has attracted worldwide attention with its spread through Western Europe and Asia in 2017, and particularly to China, the largest pork producer in the world. Several studies demonstrated that the worldwide epidemic situation may dramatically increase the risk of introduction to ASF-free countries. Currently there's no specific treatment or effective vaccine available to fight the disease. Therefore, ASF control mainly relies on classic measures, including early detection, movement control, and depopulation of the infected animals. Molecular diagnosis offers a fast and reliable tool to diagnose infectious diseases in animals. Among these, Real-time PCR assays are highly sensitive, specific and rapid and are widely used for ASFV detection around the world.

NOTE: Hi-PCR® African Swine Fever Virus (ASFV) Probe PCR Kit is for *in-vitro* use only.

Intended Use

Recommended for sensitive and specific detection of African swine fever virus in animal samples.

Principle

Real-time polymerase chain reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of PCR. This technique is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. HiMedia's Hi-PCR® African Swine Fever Virus (ASFV) Probe PCR Kit is designed to detect **all the 24 known virus genotypes in FAM channel and Internal Control in JOE channel** in a single tube reaction. The kit enables sensitive and specific detection of African swine fever virus in a single tube.

Negative Control

A negative control is needed to ensure that the reagents, equipment, and environment used in the assay is not contaminated. In this reaction, nuclease free water is used as the template.

Positive control

This is a control reaction using a known template (target pathogen). A positive control is usually used to check that the primers have been designed properly and the PCR conditions have been set up correctly.

Internal Control

This is a control sequence which is amplified in the same reaction tube along with the target sequence (target species) but detected with a different primer (i.e. Multiplex PCR). An internal

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WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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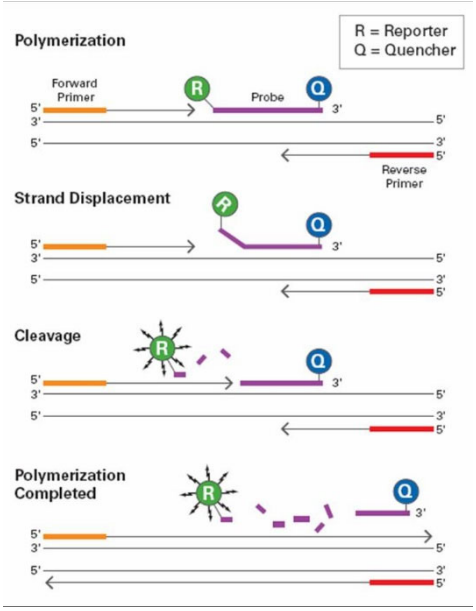
control is often used to detect the failure of amplification in cases where the target sequence is not amplified.

Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively

Strand displacement: When the probe is intact, the report dye emission is quenched.

Cleavage: During each extension cycle,the DNA polymerae cleaves the reporter dye from the probe

Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'- nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

Features

- Fast and simple
- Good sensitivity and specific results
- Guaranteed reproducible results
- Rapid detection of all relevant clinical pathogens

Types of Specimen: Blood in anticoagulant (EDTA for PCR, heparin or EDTA for virus isolation), serum, tissues mainly from spleen, lymph nodes, bone marrow, lung, tonsil and kidney. Samples should be kept as cold as possible, without freezing, during transit. After the samples arrive at the laboratory, they should be stored at –70°C if processing is going to be delayed (OIE Terrestrial Manual 2019).

Specimen collection and Handling

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with blood and other body fluids. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -10° to -20°C. Repeated thawing and freezing of PCR reagents should be avoided, as this may reduce the sensitivity. If the reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample DNA specimens can also reduce the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on pack.

Kit Contents: The provided PCR kit contains:

Components	Product code	Reagents provided for (reactions)* (µL)	
		25R	50R
Hi-Quanti 2X Realtime PCR Master Mix	MBT180	338	675
ASF Primer-Probe Mix	DS1090	27	54
Internal Control Primer-Probe Mix	DS1342	27	54
Internal Control	DS1343	540	1080
ASF Positive Control	DS1091	25	50
Molecular Biology Grade Water for PCR	ML065	200	400

*** for a 25µL reaction mix**

Materials needed but not provided:

- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19) & Sealing film (PR18)
- Insta Q Real Time PCR System (Product Code: LA1012 / LA1023 / LA1024 / LA1073/ LA1074)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes
- HiPurA® Multi-Sample DNA Purification Kit (MB554)

General Preparation Instructions

- Before use all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.

Protocol for DNA Extraction

- Specimens should be extracted before PCR amplification.
- Important: Add 20µL of internal control to every specimen at the step of adding lysis solution into the sample during extraction. Proceed further for the DNA extraction protocol as per the manufacturer's instruction manual.

When using **MB554 -HiPurA™ Multi-Sample DNA Purification Kit** for the sample extraction, follow below procedure for internal control spiking:

1. Remove Internal Control tube from Hi-PCR® African Swine Fever Virus (ASFV) Probe PCR Kit and thaw at room temperature.
2. Equilibrate the clinical specimen/tube to room temperature.
3. Add 20 µl of internal control DNA provided along with 180 µl of lysis buffer C1 (DS0010) to the sample.
4. Incubated the samples at 55°C for 10min.

5. Follow the remaining steps as mentioned in the manual of **MB554 - HiPurA™ Multi-Sample DNA Purification Kit**.

Protocol for PCR Master Mix Preparation

Components	Product Code	Volume to be added for 1R (for a 25 µL reaction)
Hi-Quanti 2X Realtime PCR Master Mix	MBT180	12.5
ASF Primer-Probe Mix	DS1090	1.0
Internal Control Primer-Probe Mix	DS1342	1.0
Molecular Biology Grade Water for PCR	ML065	5.5
Template DNA	-	5.0
Total volume	**-**	**25**

Centrifuge the tube briefly at 6000 rpm for about 10 seconds. Place the tubes in Real-time PCR machine and set the recommended PCR program (mentioned below). Interpret the data from the amplification plot (observe the Ct values).

Recommended PCR program

1. Initial denaturation : 95°C for 10 minutes
2. Denaturation : 95°C for 15 seconds
3. Annealing : 50°C for 30 seconds (Plate Read) } No. of cycles: 40
4. Plate Read : FAM/JOE
5. Hold : 4°C for ∞

Quality Control

Each lot of Hi-PCR® African Swine Fever Virus (ASFV) Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. It has been functionally tested in DNA amplification assays.

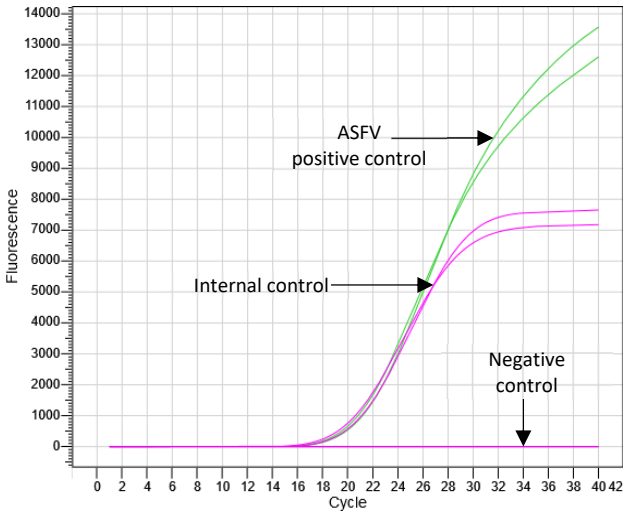
Data Analysis

The following conditions should be met for a valid diagnostic test:

Control	Detection channel	
	FAM (African Swine Fever Virus)	**JOE (Internal Control)**
Positive Control	+	+/-*
Negative Control	-	+

*The presence or absence of a signal in the JOE channel is not relevant for the validity of the test run due to competition between the test template and Internal Control template.

Amplification Data



Sr. No	Sample	C_t value
1.	ASF positive control	20.58
2.	Internal Control	20.32

Image representing probe based real-time amplification data of African Swine Fever Virus with C_t values (provided in table)

Data Interpretation

Detection Channel		Result Interpretation
FAM (AFSV)	JOE (Internal Control)	
+	+	Positive for African Swine Fever Virus
-	+	Negative for African Swine Fever Virus
-	-	PCR inhibition or reagent failure. Repeat PCR or repeat extraction from original sample

Performance Evaluation

Limit of Detection (LOD) - Analytical Sensitivity

Sensitivity for the Hi-PCR® African Swine Fever Virus (ASFV) Probe PCR Kit was conducted using synthetic DNA on InstaQ96® Real Time PCR system. The detectable limit of the Hi-PCR® African Swine Fever Virus (ASFV) Probe PCR Kit was determined to be ≈ 21 copies/reaction.

Inclusivity - Analytical Sensitivity

In silico analysis for the assessment of inclusivity for the Hi-PCR® African Swine Fever Virus (ASFV) Probe PCR Kit was conducted by mapping the primers and probes against all the available African Swine Fever Virus sequences in GenBank. The Hi-PCR® African Swine Fever Virus (ASFV) Probe PCR Kit targets 100% of the known African Swine Fever Virus strains.

Cross-reactivity - Analytical Specificity

In silico analysis was performed using NCBI nucleotide and Primer BLAST. The primers for African Swine Fever were analyzed against common swine disease pathogens (see list below). The Hi-PCR® African Swine Fever Virus (ASFV) Probe PCR Kit is specific for the 24 known virus genotypes of ASF virus.

Bovine viral diarrhea virus (BVDV)	Porcine epidemic diarrhea virus
Classical swine fever virus (CSFV)	Porcine delta coronavirus
Foot-and-mouth disease virus (FMDV)-A	Porcine parainfluenza virus
Foot-and-mouth disease virus (FMDV)-Asia1	Porcine Reproductive and Respiratory Syndrome virus

Foot-and-mouth disease virus (FMDV)-C	Porcine rotavirus
Foot-and-mouth disease virus (FMDV)-O	Seneca Valley virus USA/SSV-001
Foot-and-mouth disease virus (FMDV)-Sat1	Simian immunodeficiency virus
Foot-and-mouth disease virus (FMDV)-Sat2	Transmissible Gastroenteritis Virus
Foot-and-mouth disease virus (FMDV)-Sat3	Vesicular stomatitis virus (VSV)

Warning and Precautions

Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in under quantitation or failure to detect the presence of the target regions in these cases. Validity and performance of the assay design are revised at regular intervals.

Performance and Evaluation

Each lot of Hi-PCR® African Swine Fever Virus (ASFV) Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	1. Check the integrity of DNA using agarose gel electrophoresis.
			2. Use freshly prepared DNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Verify that the correct reagent volumes, dilutions and storage conditions have been used.
2.	Variability between replicates	Error in reaction set-up	Prepare a large volume master mix, vortex thoroughly and aliquot into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a real-time PCR instrument.
		Pipetting error	C_t values of replicates can show increased variation due to poor laboratory technique or imprecise pipettes.
3.	Amplification in negative control	Reagents contaminated	1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.
4.	No signal with positive controls	Incorrect programming of the temperature profile of the thermocycler	Compare the temperature profile to the manual.

Safety Information

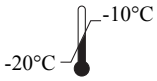
Hi-PCR® African Swine Fever Virus (ASFV) Probe PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.



Storage temperature

Do not use if package is damaged

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03/2027

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