TargetEx ASFV qPCR Diagnostic Kit

Catalogue Number: TGX-044-01

INTRODUCTION

Our **multiplex** TargetEx ASFV qPCR Diagnostic Kit is designed for the detection of African Swine Fever (ASF) virus DNA by quantitative PCR. In addition to the ASF virus-specific FAM™-labelled probe, the assay is capable of detecting endogenous controls (e.g., swine, boar or warthog DNA, probe labelled with HEX™) as well as exogenous controls (probe labelled with Cy5™), ensuring both sample integrity and assay performance.

The detection process is completed in **under 50 minutes** using the standard protocol, while a **rapid protocol** is also available, reducing the experiment time to **45 minutes**.

The kit is compatible with a **wide range of sample** types, including serum, whole blood, various organs (e.g., liver, brain, intestine), bone marrow and other tissue samples. You can pool up to 3–5 samples (recommended in case of high DNA concentration only).

The kit includes both positive and negative ASFV controls, each producing an expected Cq value of approximately 28 during amplification for ASFV and endogenous target. Additionally, it contains an extraction (exogenous) control designed to monitor the efficiency and reliability of the DNA extraction process. Please note, that the extraction control can be utilized in two distinct ways depending on your DNA extraction method:

A. It can be added as a spike-in to the sample **prior to extraction** (extraction spike-in).

B. It can also be added **directly to the extracted DNA** sample to verify the PCR reaction itself. In this case, a highly diluted control should be used to avoid interference with the assay (**details in the setup instructions below**).

Store the kit components at -20°C.
Avoid freeze-thaw cycles.

KIT COMPONENTS

Reagent	Description	Volume (µL)
Reaction Mix (100 reactions)	Master Mix includes primers and probes	1500*
Positive ASFV Control (PC)	ASFV-specific target DNA	50
Negative ASFV Control (NC)	Endogenous target DNA	50
Extraction Control (EC)	Exogenous target DNA	50

* The reaction mix includes a 10% excess (final volume: 1650 µL) to ensure compatibility with automated liquid handling systems.

REQUIRED MATERIALS AND EQUIPMENT (NOT PROVIDED)

- Nuclease-Free Water
- Vortex
- Centrifuge
- Pipettes and filtered tips (nuclease-free)
- Sterile microcentrifuge tubes (1.5 mL)
- 96-well PCR plate/PCR strip with adhesive film/cap
- Real-Time PCR thermo-cycler (with FAM, HEX and Cy5 channels)

SETUP INSTRUCTIONS

CONTROLS

For valid interpretation please use the recommended controls in your assay (see Controls summary table).

For reliable results perform replicates of all reactions. Keep the kit components on ice during preparation.

Extraction Control Application
The method of application depends on the DNA extraction workflow:

A. Pre-extraction spike-in: **Add 1 µL** (1 ng) of the extraction control directly to the sample before DNA extraction.
B. Post-extraction addition: Add the extraction control directly to the **extracted DNA** in **10^{-5} ng/µL final concentration**.

REACTION SETUP

1. Thaw all components of the ASF Virus Diagnostic Kit in a sterile hood.
2. Gently vortex each tube and briefly centrifuge.
3. Add **15 µL of Reaction Mix** to each well designated for analysis.
4. Add **5 µL template DNA** (typically 0.5-25 ng per reaction) or **5 µL** of the **Positive** or **Negative ASFV Control** or **5 µL Nuclease-Free Water (NTC)** to each well or strip. Mix gently by pipetting. (A multichannel pipette can be used for this step.) Change pipette tips between wells!
5. Seal the plate or strip using adhesive film or suitable caps.
6. Briefly centrifuge the plate or strip.
7. Place the plate/strip into the qPCR thermal cycler and initiate the PCR program with the parameters listed below.

THERMAL CYCLER PROGRAM SETUP

No.	Step	Temperature (°C)	Duration
1	Initial Denaturation	95	3 min
2	Denaturation (per cycle)	95	5 sec
3	Annealing/Extension (per cycle)	60	10 sec

Repeat step 2 and 3 39 times (total of 40 cycles) with fluorescence detection at the end of each Annealing/Extension step.
Optional: 5 sec Annealing/Extension time can be used in the rapid protocol. This method is only suggested with high quality DNA samples (260/280 absorbance ratio ~1.8).

CONTROLS SUMMARY TABLE

Control Type	Target	Channel	Expected Cq Value
Positive ASFV Control (PC)	Verifies ASFV-specific amplification	FAM	≤ 30
Negative ASFV Control (NC)	Verifies endogenous target amplification, works as internal control (IC) for ASFV	HEX	≤ 30
Extraction Control (EC)	Monitors extraction and PCR efficiency	Cy5	25–35*
No Template Control (NTC)	Excludes reagent contamination	–	No amplification

* Expected Cq value varies depending on the DNA extraction method, sample type, etc.

EVALUATION

ASSAY VALIDATION

Before interpreting the results, make sure the following criteria are met:

- True amplification is indicated exclusively by sigmoidal amplification curves.
- Set the threshold in the linear range of the exponential amplification curve (for more details follow the guidelines of the instrument's manufacturer).
- NTC: No amplification detected in no-template control.
- PC: The positive ASFV control should amplify in the FAM channel with a Cq value ≤ 30.
- NC: The negative ASFV control is expected **not to amplify** in the FAM channel but should amplify in the HEX channel with a Cq value ≤ 30.
- EC: The Cq value of the extraction control varies depending on the extraction method and sample type; if the Cq value is ≥ 35, repeating the extraction with increased amount of extraction control is recommended.
- If the Cq value of your test sample is above 35 cycles, consider repeating the experiment using higher amount of DNA and additional controls.

SUGGESTED INTERPRETATION OF DATA

After verifying your assay, use the table below for data interpretation:

Result	ASF Virus (FAM)	Endogenous control (HEX)	Exogenous control (Cy5)	Conclusion
Positive	+	+	+	ASFV detected.
Positive (IC failure)	+	–	+	ASFV detected; but endogenous control failed – insufficient sample quality, repeat extraction/qPCR protocol.
Positive (EC failure)	+	+	–	ASFV detected; but exogenous control failed – repeat extraction with higher EC concentration.
Positive (IC and EC failure)	+	–	–	ASFV detected but both endogenous and exogenous control failed – repeat extraction with higher EC concentration.
Negative	–	+	+	ASFV not detected.
Negative (IC failure)	–	–	+	Endogenous control failed – insufficient sample quality, repeat extraction/qPCR protocol.
Negative (EC failure)	–	+	–	Exogenous control failed – repeat extraction with higher EC concentration.
Invalid	–	–	–	No amplification of target or controls – repeat full extraction and qPCR workflow.

If you need further information or have any questions, please don't hesitate to email us at **info@targetex.com**!