# African swine fever virus dual nucleic acid detection Kit (MIRA-exo) Guide Manual

**（Part number：WLP9250KIT）**

## Product description

The kit designs specific primers and probes in the highly conserved regions of the P72 gene and MGF360-10L gene of African swine fever virus(ASFV), respectively. It can detect the African swine fever virus(ASFV) P72 gene and MGF360-10L gene in swine blood, serum, or tissues in 20 minutes by MIRA technology.

## Overview of the MIRA technology

Multienzyme isothermal rapid amplification(MIRA) is based on a combination of polymerases and DNA recombination/repair proteins: Recombinases bind to single-stranded nucleic acid and stimulate the resulting protein-DNA complex to search for homologous sequences in duplex DNA with Single Strand Binding protein. Once homology is located,a strand-switching reaction is performed and the oligonucleotide is paired to its complement permitting a polymerase to begin synthesis from the 3’ end. The amplification process is very rapid when optimised and can reach detectable levels of product in a few minutes.

## Materials provided

| Materials provided | Content |
|---|---|
| **E Buffer** | 2 tube × 1 mL |
| **B Buffer** | 1 tube × 150 μL |
| **Positive control DNA template** | 1 tube × 100 μL |
| **Steel bead reagent** | 48 |
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## Storage conditions

- Transport temperature: ≤ 20 ºC.
- Store at -20 ºC without light，no repeated freezing and thawing.
- Full activity is guaranteed for 14 months.

## Set up

1. Thaw reagents at room temperature.
2. Shake the lyophilized powder tube to confirm that each tube has a small steel bead.Thoroughly mix the contents of each tube by vortexing, then briefly centrifuge.
3. Add 37.5 μL E buffer (supplied) to a sample response MIRA exo reaction.
4. Add 10μL sample DNA.

5. Add 2.5 μl B buffer (supplied) and mix well and spin briefly to start reaction（For multiple reactions, it is recommended to add B buffer to the inner side of the reaction tube cover, and turn the reaction tube upside down to mix well）.

6. Turn on the Amp Future WL-16-II Constant Temperature Fluorescence Detector, put the reaction tube into the device and record the sample placement sequence, select DNA mode, 16-well synchronization, and both of FAM/VIC channel. The amplification temperature of this program was 39 - 42 ºC, 30 s/cycle, 20 minutes, and a total of 40 cycles.

## Reaction mix

| | |
|---|---|
| E Buffer | 37.5 μL |
| DNA sample | 10 μL |
| B Buffer | 2.5 μL |
| Total volume | 50 μL |

## Interpretation of results

1. MGF360-10L gene positive:the amplification curve of FAM channel is S-like, and the instrument is judged as "+".

2. 2. P72 gene positive:the amplification curve of VIC channel is S-like, and the instrument is judged as "+".

3. Negative: the instrument both of FAM/VIC channel is judged as "-".

Note: When affected by the color of the sample or other factors, the machine shows the result as "+" but there is no "S"-shaped amplification curve, the result is judged as negative.

## Note

1. Reactions start as soon as B buffer is added.
2. Set up a negative control for each experiment.
3. For research and development use only.

## Ordering information and technical support

Amp-Future (Changzhou) Biotech Co., Ltd.
4th Floor, No.9 Building, China Israel Changzhou Innovation Park (CICP),
No.18-67 Changwu Mid Road,
Wujin District
Changzhou City
Jiangsu Province
China

sale@amp-future.com
Tel: 0519-88558626