

VetScreen PRRSV (American Mutant)

User Manual
Cat. No. V03-01-1125

One-step reverse transcription PCR for gel-based detection of Porcine Respiratory and Reproductive Syndrome Virus American Mutant Subtype in veterinary and environmental samples

Includes main components for 50 reactions



Rev. 1
July 2009

HAI KANG LIFE CORPORATION LIMITED

PLEASE READ THROUGH THE ENTIRE PROTOCOL BEFORE STARTING.

1. KIT COMPONENTS

DNA Amplification Reagents

- 2 x 500 µl PRRSV Mutant Mastermix (store at -20°C)
- 2 x 25 µl Positive Control (store at -20°C)
- 1 x 25 µl Taq Polymerase (store at -20°C)

Storage Conditions

Store Mastermix at -20°C. Thaw frozen reagents just before use. Mix reagents thoroughly (do not vortex mastermix containing enzyme).

Note:

Please aliquot the Mastermix into appropriate volume according to your test frequency, in order to minimize repeated freeze and thaw cycles. Frequent thawing and freezing may inactivate some kit components.

2. PROCEDURES

Set-up

- In addition to the RNA obtained from the test samples, each experiment requires a positive and negative (water) control.
- Set up PCR components according to the table below:

Components	Volume per reaction
Mastermix	19.5 µl
Taq Polymerase	0.5 µl
Sample RNA	5 µl
Total Volume	25 µl

Note:

- 5 µl of the provided Positive Control can be used to monitor the success of amplification.
- Keep RNA samples on ice throughout experiment.
- It is advisable to run samples in duplicate to ensure reliability of results.
- Spiking Control: 1 µl Positive Control can be spiked into test sample to check whether the test sample contains PCR inhibitory substances.

Cycling conditions:

1 Cycle	42°C	30 Minutes
	95°C	10 Minutes
40 Cycles	95°C	30 Seconds
	53°C	30 Seconds
	72°C	30 Seconds
	72°C	30 Seconds

3. DATA ANALYSIS AND INTERPRETATION

The PCR products were analysed using 2% agarose gel electrophoresis and expected wild type strains PCR product size 330 base pairs while mutant strains PCR product size 300 base pairs.

Spiking Control

Negative PCR result may be due to a few scenarios: 1. absence of detected sequence in the sample; 2. presence of detected sequence below limit of detection; 3. presence of PCR inhibitory substances. The purpose of spiking control is to verify whether the test sample contains substances, which may affect PCR reactions. If no band is visible on agarose gel when the test sample is spiked with the positive control, the test sample is highly likely to contain PCR inhibitory substances, and the result should NOT be taken as negative. Repeated extraction and PCR of the sample will be required.

If you require more detailed analysis information, please contact Hai Kang Life Corporation Limited for technical assistance.

4. TECHNICAL ASSISTANCE

Our technical staff will provide technical assistance you may need in using this kit. Simply call +(852) 2111 2123 during office hours:

Monday – Friday: 9:00am to 5:30pm

Saturday: 9:00am to 1:00pm

A recorded message (in English, Cantonese or Putonghua) may be left outside office hours. Alternatively, you may contact our technical staff by fax or email.

Fax: +(852) 2111 9762

Email: technical@haikanglife.com

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