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INDUSTRY STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

SN/T 3327-2012

Quarantine protocal for classic swine fever virus by loop-mediated isothermal amplification

猪瘟病毒逆转录环介导等温核酸扩增检测方法

(English Translation)

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Foreword

This standard was drafted in accordance with GB/T 1.1-2009 given rules.

This standard is proposed and managed by the National Certification and Accreditation Administration Committee.

This standard was drafted. People's Republic of China Shanghai Entry-Exit Inspection and Quarantine Bureau.

1 Scope

This standard specifies the method of operation of classical swine fever virus by reverse transcription loop-mediated isothermal nucleic acid amplification detection.

This standard applies to rapid screening of classical swine fever virus.

2 Normative references

The following documents for the application of this document is essential. For dated references, only the dated version suitable for use herein Member. For undated references, the latest edition (including any amendments) applies to this document.

GB/T 18088 Entry and Exit Animal Quarantine sampling GB 19489-2008 General requirements for laboratory biosafety

3 Overview

Classical swine fever (classicalswinefever, CSF), is a kind of emergency by the classical swine fever virus (classicalswinefevervirus, CSFV) caused Resistance, heat resistance, highly contagious disease of swine breeding industry very serious harm. CSFV genotype positive strand RNA, genome about 123kb, contains only one large open reading frame (ORF). The standard use of loop-mediated isothermal nucleic acid amplification at a constant temperature Fast, efficient and specifically amplify located fever virus 5

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'untranslated region (5'-UTR) of the target sequence, so as to achieve rapid detection purposes.

4 Reagents and materials

4.1

BstDNA polymerase (large fragment). enzyme concentration was 8U/μL, -20 °C, avoid repeated freezing and thawing.

4.2 10 times ThermoPol polymerase buffer concentrate. -20 °C to save.

4.3

magnesium sulfate (MgSO4). concentration of 25mmol/L, -20 °C preservation.

4.4

AMV reverse transcriptase. reverse transcriptase concentration of 5U/μL, -20 °C, avoid repeated freezing and thawing.

4.5

betaine solution (Betaine). concentration 5mol/L, -20 °C, avoid repeated freezing and thawing.

4.6

dNTPs. containing dATP, dGTP, dCTP, dTTP each 10mmol/L, -20 °C preservation.

4.7

LAMP primer. dubbed the concentration of 25μmol/L, -20 °C stored for use. Its sequence is as follows.

--- Being outwardly primer (CSFV-F3). 5'-AGC-TCC-CTG-GGT-GGT-CTA-3 ';

--- Reverse outer primer (CSFV-B3). 5'-CCT-AAT-AGT-GGG-CCT-CTG-CA-3 ';

--- Within the forward primer (CSFV-FIP).
5'-GCC-CTC-GTC-CAC-ATA-GCA-TCT-CAG-TAC-AGG-ACA-GTC-GTC-AGT-3 ';

--- Within the reverse primer (CSFV-BIP).
5'-GCC-CAA-GAC-ACA-CCT-TAA-CCC-TTC-AGG-TCG-TAC-TCC-CAT-CAC-3 ';

--- Forward loop primer (CSFV-LF). 5'-GGG-CTT-CTG-CTC-ACG-TCG-3 ';

--- Reverse loop primer (CSFV-LB). 5'-GGT-CGC-TAG-GGT-GAA-ATC-3 '.

4.8

SYBRGreenI fluorescent dye solution. -20 °C to save.

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