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SN

**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

SN/T 1199-2010

Replacing SN/T 1199-2003

**Protocol of the polymerase chain reaction for detecting
genetically modified components in cotton**

棉花中转基因成分定性PCR检验方法

(English Translation)

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Foreword

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

Instead of the standard SN/T 1199-2003 "Genetically Modified Cotton Qualitative PCR detection method." this standard compared with SN/T 1199-2003, the main technical changes are as follows.

- Standardized terminology and way of expression;
- Increase the range "of genetically modified cotton line MON531, MON1445 and real-time PCR strains MON15985 Identification ";
- Terminology and abbreviations reference GB/T 19495.1 "GMO testing laboratories technical requirements" provisions;
- Of cotton DNA extraction, using some of CTAB method (2003 version 6.4.1 and 6.4.2; 9.1.1 Excerpts) mining Method GB/T 19495.3 prescribed; 9.1.2 Where the increase of the sample is not available for the method in accordance with 9.2.1 GB/T 19495.3 specified in the appropriate way to do;
- Food in nucleic acid quantification (2003 Version 6.5; 9.2 edition) using the method GB/T 19495.3 prescribed;
- Increasing the real-time PCR method, and the result is determined in step (2003 edition, Chapter 7; edition 9.5.2) corresponding increase Plus real-time PCR assay results to determine the method;
- Conventional PCR amplification reaction system, the reaction cycle number and the result of the judgment (2003 edition 6.6.1, 6.6.2, 6.6.3 Table 2 and Table 3; Excerpts 8.5.1, Table A.1, Table A.3 and Table A.4) way of expression to rearrange and modify the confirmatory test method.

This standard is proposed and managed by the National Certification and Accreditation

Administration Committee.

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

1 Scope

This standard specifies the qualitative PCR detection of genetically modified cottonseed.

This standard applies to cotton seed and semi-finished products in the anti-lepidopteran cotton SGK321, SGK9708, GK1, GK2, GK3, GK5, GK12, GK19, GK22, GK95-1 GMO ordinary PCR screening assays and transgenic cotton line MON531, MON1445 Realtime PCR and MON15985 strain identification.

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, undated references, the latest edition (including any amendments) applies to this document.

GB 5491 grain and oilseeds testing for sampling, and sample method GB/T 6682 analytical laboratory use specifications and test methods GB/T 10360 for sampling oilseed residues GB/T 19495.1 GMO testing - General requirements and definitions GB/T 19495.2 technical requirements for GMO testing laboratories GB/T 19495.3-2004 GMO detection of nucleic acid extraction and purification method

3 Terms and abbreviations

Terms and abbreviations GB/T 19495.1 defined apply to this document.

Principle 4

After extraction of the sample DNA, for GM cottonseed exogenous gene designed primer and probe sequences by conventional PCR, special Specifically amplify DNA fragments of exogenous gene, and based on PCR determine whether the sample contains genetically modified ingredients. To carry out strain KAM Given, through the real-time PCR technology to determine whether the sample contains a strain.

5 Apparatus appliances

5.1

cycler, electrophoresis, tank, gel imaging analysis system, UV-visible spectrophotometer, balance (and a sense of the amount of 1mg 0.1mg), autoclaves, cryogenic refrigerators, refrigerated centrifuge, water bath, microwave, real-time PCR instrument, clean benches, Ultra-pure water, Vortex.

5.2

micro pipette (2.5 μ L, 10 μ L, 20 μ L, 100 μ L, 200 μ L, 1000 μ L), PCR reaction tube (200 μ L, 500 μ L), Eppendorf tubes (1.5mL and 2.0mL).

6 Reagents

Unless otherwise specified, other reagents were of analytical grade or biochemical reagents, water as a water according to GB/T 6682 regulations.