GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

ICS 07.080

A 40

GB/T 38505-2020

General detection methods of genetically modified products

转基因产品通用检测方法

Issued on: March 6, 2020 **Implemented on: March 6, 2020**

Issued by: State Administration for Market Regulation;
Standardization Administration of PRC.

Table of Contents

General testing method for genetically modified products

1 Scope

This standard specifies the qualitative testing methods for genetically modified products.

This standard is applicable to the general testing of genetically modified components in rice, corn, soybean, rapeseed, potato, sugar beet, alfalfa, and their processed products by real-time fluorescent PCR.

The minimum detection limit of the method of this standard is 0.1% (mass fraction).

2 Normative references

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this standard.

GB/T 6682 Water for analytical laboratory use - Specification and test methods

GB/T 19495.1 Detection of genetically modified organisms and derived products - General requirements and definitions

GB/T 19495.3 Detection of genetically modified organisms and derived products - Nucleic acid extraction

GB/T 19495.7 Detection of genetically modified organisms and derived products - Methods for sampling and sample preparation

3 Terms and Definitions

The following terms and definitions apply to this document.

3.1

18S rRNA gene

Transcribed from 18S rDNA, one of the components of the ribosome, which exists as a plant endogenous gene.

3.2

35S promoter from *Cauliflower mosaic virus*

The 35S promoter that is from the cauliflower mosaic virus (CaMV).

3.3

35S terminator from *Cauliflower mosaic virus*

The 35S terminator that is from the cauliflower mosaic virus (CaMV).

3.4

terminator of nopaline synthase gene

The terminator that is from the nopaline synthase gene.

3.5

terminator of ribulose-1,5-biphosphate carboxylase small subunit

It is from the 3' terminator sequence of the ribulose-1,5-biphosphate carboxylase small subunit gene of peas.

3.6

phosphinothricin acetyltransderase gene

It is from the *Streptomces viridochromogenes* and encoded as phosphinothricin acetyltransderase (PAT).

Note: The *pat* gene is tolerant to the herbicide “glyphosate”.

3.7

terminator of proteinase inhibitor II

The terminator that is from the proteinase inhibitor II (*Pin* II) of potatoes.

3.8

promoter of ribulose-1,5-biphosphate carboxylase small subunit 1A

The promoter that is from the encoding gene of the ribulose-1,5-biphosphate carboxylase small subunit 1A (*RbcS4*) of the Arabidopsis thaliana.

3.9

5'-end event-specific sequence of DAS40278

The sequence of the junction region between the 5' end of the exogenous insert of DAS40278 transformant and the maize genome.

Note: This sequence includes part of the vector sequence of the exogenous insert and part of the sequence of the maize genome.

3.10

3'-end event-specific sequence of DP305423

The sequence of the junction region between the 3' end of the exogenous insert of DP305423 transformant and the soybean genome.

Note: This sequence includes part of the vector sequence of the exogenous insert and part of the sequence of the soybean genome.

3.11

5'-end event-specific sequence of CV127

The sequence of the junction region between the 5' end of the exogenous insert of CV127 transformant and the soybean genome.

Note: This sequence includes part of the vector sequence of the exogenous insert and part of the sequence of the soybean genome.

3.12

cycle threshold

The number of cycles through which the fluorescent signal in the reaction tube reaches the set threshold.

4 Reagents and materials

Unless otherwise specified, all reagents shall be analytical reagents or biochemical reagents. The water used in the test shall meet the specifications of Grade 1 water in GB/T 6682.

4.1 Real-time fluorescent PCR premix solution

Use a real-time fluorescent PCR premix solution that has been verified to meet the requirements of real-time fluorescent PCR.

4.2 500 mmol/L ethylenediaminetetraacetic acid disodium solution (pH 8.0)

Carry out the operation in accordance with the provisions of GB/T 19495.1 and GB/T 19495.3.

6.4 DNA template preparation

Carry out the operation in accordance with the provisions of GB/T 19495.1 and GB/T 19495.3. Alternatively, DNA template preparation can be performed by using a plant genomic DNA extraction kit with the same effect.

6.5 DNA concentration determination

Determine the DNA concentration with UV spectrophotometry; dilute the DNA solution appropriately, measure its absorbance at 260 nm, and calculate the DNA concentration according to the measured OD (at 260 nm, 1 OD=50 μg/mL double-stranded DNA); the OD shall be in the range of 0.2~0.8. Measure its absorbance at 280 nm, and calculate the $OD_{260\ nm}/OD_{280\ nm}$ ratio of the DNA solution according to the measured OD; the ratio shall be in the range of 1.8~2.0.

6.6 Real-time fluorescent PCR detection

6.6.1 Setting of negative control, positive control, and blank control

Take the non-transgenic sample as the negative control; take the corresponding transgenic plant sample strain, or the genomic DNA of the transgenic plant sample containing the corresponding exogenous gene, or the plasmid standard molecular DNA containing the above fragments as the positive control; take the water or TE buffer as the blank control.

6.6.2 Real-time fluorescent PCR reaction system

See Table 3 for the PCR reaction system, or prepare it according to the recommended system of a kit that has been verified to meet the requirements. Make 2 parallel tubes for each DNA sample. When the sample is added, the sample DNA solution shall be completely added to the reaction solution, and it shall not stick to the tube wall. After the sample is added, the tube cap shall be tightly closed as soon as possible.

Blank control: No typical amplification curves appear in the detection of the internal reference gene, and no typical amplification curves appear in the detection of all exogenous genes, or the cycle threshold is greater than or equal to 40.

Negative control: A typical amplification curve appears in the detection of the internal reference gene, and the cycle threshold is less than or equal to 30; no typical amplification curves appear in the detection of all exogenous genes, or the cycle threshold is greater than or equal to 40.

Positive control: A typical amplification curve appears in the detection of the internal reference gene, and the cycle threshold is less than or equal to 30; a typical amplification curve appears in the detection of all exogenous genes, and the cycle threshold is less than or equal to 34.

8 Judgment and presentation of results

8.1 Result judgment

All the test samples are tested in parallel, if no typical amplification curves appear in the exogenous gene detection, or the cycle threshold is greater than or equal to 40; and if a typical amplification curve appears in the endogenous gene detection and the cycle threshold is less than or equal to 30, then it can be determined that the sample does not contain the tested exogenous genes.

All the test samples are tested in parallel, if a typical amplification curve appears in the exogenous gene detection and the cycle threshold is less than or equal to 36, and if a typical amplification curve appears in the endogenous gene detection and the cycle threshold is less than or equal to 30, then it can be determined that the sample contains the corresponding exogenous gene.

All the test samples are tested in parallel, if a typical amplification curve appears in the exogenous gene detection, but the cycle threshold is in the range of 36~40; and if a typical amplification curve appears in the endogenous gene detection and the cycle threshold is less than or equal to 30, then the sample shall be reprocessed and tested again after exclusion of contamination. After re-amplification, if a typical amplification curve appears in the endogenous gene detection and the cycle threshold is less than or equal to 30, and if a typical amplification curve appears in the exogenous gene detection and the cycle threshold is still less than 40, then it can be determined that the sample contains the tested exogenous genes. After re-amplification, if a typical amplification curve appears in the endogenous gene detection and the cycle threshold is less than or equal to 30, and if no typical amplification curves appear in the exogenous gene detection, or the cycle threshold is greater than or equal to 40, then it can be determined that the sample does not contain the tested exogenous genes.

This is an excerpt of the PDF (Some pages are marked off intentionally)

------ The End ------