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

SN/T 5334.7-2020

**Digital PCR detection method for genetically modified plant
products - Part 7: Genetically modified alfalfa**

转基因植物产品的数字PCR检测方法 第7部分：转基因苜蓿

(English Translation)

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Foreword

SN/T 5334-2020 "Digital PCR Detection Method for Genetically Modified Plant Products" is divided into 8 parts.

- Part 1: General requirements and definitions;
- Part 2: Genetically modified soybeans;
- Part 3: Genetically modified corn;
- Part 4: Genetically modified rape;
- Part 5: Genetically modified cotton;
- Part 6: Genetically Modified Potatoes;
- Part 7: Genetically modified alfalfa;
- Part 8: Genetically modified sugar beet.

This part is part 7 of SN/T 5334-2020.

This document was drafted in accordance with the rules given in GB/T 1.1-2020.

Certain contents of this document may involve patents. The issuing agency of this document is not responsible for identifying these patents.

This document was proposed and managed by the General Administration of Customs of the People's Republic of China.

1 Scope

This document specifies the quantitative detection method of genetically modified strains specific to imported and exported alfalfa by digital PCR (dPCR).

This document is suitable for the quantitative detection of specific components of genetically modified alfalfa J101, J163, and KK179 strains in alfalfa seeds and alfalfa grass.

The lower limit of quantitative detection in this document is 7.0 copies/ μ L, and the lower limit of qualitative detection is 1.4 copies/ μ L.

2 Normative references

The contents of the following documents constitute the indispensable clauses of this document through normative references in the text. Among them, dated quotations Only the version corresponding to that date is applicable to this document; for undated references, the latest version (including all amendments) is applicable Used in this document.

SN/T 5334.1 Digital PCR Method for Quantitative Detection of Genetically Modified Products Part 1: General Requirements and Definitions

3 Terms and definitions

The terms and definitions defined in SN/T 5334.1 apply to this document.

4 Principles of the method

See SN/T 5334.1.

5 Equipment and reagents

5.1

Endogenous gene and exogenous gene. the internal standard gene is acetyl-coenzyme A carboxylase gene (ACC); the exogenous sequence is strain J101, J163 and KK179 foreign gene insertion site 5'end cross-border sequence. Primers for dPCR quantitative detection of genetically modified strains in alfalfa See Appendix A for the probe sequence.

5.2

For other equipment and reagent requirements, please refer to SN/T 5334.1.

6.1 General operation steps

For general operation steps, see SN/T 5334.1.

6.2 Digital PCR reaction system

Prepare the final reaction system of digital PCR according to Table 1.

- a. It is recommended to use the digital PCR platform currently on the market for experiments;
- b. The types of reagents added in the final reaction system and the total system volume may vary according to different digital PCR platforms. Perform different digital PCR when configuring the system of the platform, it is necessary to refer to the instructions of the digital PCR instrument of different platforms, and it is necessary to ensure that the final concentration of the components listed in Table 1 remains unchanged.

6.3 Digital PCR reaction program

6.3.1

Perform digital PCR amplification in accordance with the procedures in Table 2.

- a. According to the requirements of different digital PCR platforms, the hot start step in the reaction program can be modified, but the thermal cycling (amplification) step must not be modified.
- b. According to the requirements of different digital PCR platforms, it is possible to perform post-processing after the thermal cycling (amplification) step. The post-processing steps are different Digital PCR platform instructions were carried out.

6.3.2

For other requirements, see SN/T 5334.1.

7 Judgment and expression of results

See SN/T 5334.1 for calculation of results.

- Both the internal standard gene and the foreign gene in the test result meet the negative and blank quality control requirements, indicating that no alfalfa has been detected the endogenous gene ACC is expressed as "alfalfa component not detected";
- In the test results, the internal standard gene result is positive, but the foreign gene meets the quality control requirements of negative and blank.
Genetically modified strains";
- In the test results, both the internal standard gene and the exogenous genome meet the

quality control requirements of the digital PCR test of the sample, indicating that

8 Anti-pollution measures

The anti-pollution measures are specified in SN/T 5334.1.

9 Sample storage

For sample storage, see SN/T 5334.1.

Appendix A

(Informative appendix) Note. The underlined part is the alfalfa genome sequence, the shaded part is the upstream and downstream primer sequence, and the boxed part is the probe sequence A.4 Primer and probe sequence See Table A.1.