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Horizontal methods for molecular biomarker analysis — Methods of analysis for the detection of genetically modified organisms and derived products —

Part 9: Construct-specific real-time PCR based screening method for the detection of the P35S-*npt*II DNA-sequence

Méthodes horizontales pour l'analyse moléculaire de biomarqueurs — Méthodes d'analyse pour la détection des organismes génétiquement modifiés et des produits dérivés —

*Partie 9: Méthode de criblage construit spécifique basée sur la PCR en temps réel pour la détection de la séquence ADN P35S-*nptII

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Contents

Page

Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

A list of all parts in the ISO 21569 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Horizontal methods for molecular biomarker analysis — Methods of analysis for the detection of genetically modified organisms and derived products —

Part 9: Construct-specific real-time PCR based screening method for the detection of the P35S-*npt*II DNA-sequence

1 Scope

This document specifies a procedure for the detection of the DNA transition sequence between the 35S promoter region from cauliflower mosaic virus (P35S) and the neomycin-phosphotransferase gene (*npt*II) from the Tn5 transposon of *Escherichia coli*. The P35S-*npt*II segment is part of a construct which confers resistance to neomycin/kanamycin antibiotics frequently found in genetically modified (GM) plants.

The detection method is based on real-time PCR and can be used for qualitative screening purposes. For identification and quantification of a specific GM plant (event) a follow-up analysis has to be carried out.

This method is applicable for the analysis of DNA extracted from foodstuffs. It can also be suitable for the analysis of DNA extracted from other products such as feedstuffs and seeds. The application of this method requires the extraction of an adequate amount of amplifiable DNA from the relevant matrix.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 16577, *Molecular biomarker analysis — Vocabulary for molecular biomarker analytical methods in agriculture and food production*

ISO 21569, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Qualitative nucleic acid based methods*

ISO 21571, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Nucleic acid extraction*

ISO 24276, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 16577 apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Principle

DNA is extracted from the test sample by applying a suitable method (see ISO 21571). The DNA analysis consists of two parts:

a) verification of the amount and amplifiability of the extracted DNA, e.g. by means of a simplex real-time PCR specific for the universal plant actin gene[1][2] or another target taxon (see ISO 21570);

b) detection of the P35S-*npt*II DNA sequence in a simplex real-time PCR.[4][5]

NOTE In the case of a positive result, further analyses, if possible, with event-specific PCR methods, are carried out for the identification of the GM plant (event).

5 Reagents and materials

5.1 General

Chemicals of recognized analytical grade, appropriate for molecular biology shall be used, as a rule. The water used shall be double distilled or PCR grade water, i.e. nuclease and nucleic acid free. For all operations in which gloves are used, it should be ensured that these are powder-free. The use of aerosol-protected pipette tips as protection against cross-contamination is recommended.

5.2 PCR reagents

5.2.1 Thermostable DNA polymerase (for hot-start PCR).

5.2.2 PCR buffer solution, containing magnesium chloride and deoxyribonucleoside triphosphates (dNTPs).

Ready-to-use reagent mixtures or mixes of individual components can be used. Reagents and DNA polymerases that lead to equal or better results may also be used.

5.2.3 Oligonucleotides (see Table 1).

Equivalent reporter dyes and/or quencher dyes may be used for the probe if they can be shown to yield similar or better results.

Table 1 — Oligonucleotides

Name	DNA sequence of the oligonucleotide	Final concentration in the PCR
Plant actin as the target sequence[1][2]:		
Act-f	5′- CAA gCA gCA TgA AgA TCA Agg T –3′	900 nmol/l
Act-r	5′- CAC ATC TgT Tgg AAA gTg CTg Ag -3′	900 nmol/l
Act-probe	5′-(FAM)- CCT CCA ATC CAg ACA CTg TAC TTY CTC TC -(BHQ1)-3′	200 nmol/l
P*35S-nptII* construct as the target sequence[4][5]:		
Primer 35S-F	5′- TAT CCT TCg CAA gAC CCT TCC -3′	400 nmol/l
Primer *npt*II-R	5′- gAT TgT CTg TTg TgC CCA gTC A -3′	400 nmol/l
Probe *npt*II-Tm2	5′-(FAM)- AgC CgA ATA gCC TCT CCA CCC AAg C -(BHQ1)-3′ [a]	100 nmol/l

Key

FAM: 6-Carboxyfluorescein; BHQ®1: Black Hole Quencher 1.

NOTE BHQ®1 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.