



BSI Standards Publication

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

Part 4: Real-time PCR based screening methods for
the detection of the *P-nos* and *P-nos-nptII* DNA
sequences

National foreword

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

Part 4: Real-time PCR based screening methods for the detection of the *P-nos* and *P-nos-nptII* DNA sequences

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

*Partie 4: Méthodes de dépistage PCR en temps réel pour la détection
des séquences ADN P-nos et P-nos-nptII*



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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 documents 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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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

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

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

Part 4:

Real-time PCR based screening methods for the detection of the *P-nos* and *P-nos-nptII* DNA sequences

1 Scope

This document specifies a procedure for the detection of a DNA sequence of the promoter region of the nopaline synthase gene (*P-nos*) from *Agrobacterium tumefaciens* and a procedure for the detection of the DNA transition sequence between *P-nos* and the neomycin-phosphotransferase gene (*nptII*) from the Tn5 transposon of *Escherichia coli* K12. The *nos*-promoter and the *P-nos-nptII*-construct are frequently found in genetically modified plants. The *P-nos* and *P-nos-nptII* specific methods are based on real-time PCR and can be used for qualitative screening purposes. For identification and quantification of a specific genetically modified plant (event) a follow-up analysis has to be carried out.

The methods described are applicable for the analysis of DNA extracted from foodstuffs. They may also be suitable for the analysis of DNA extracted from other products such as feedstuffs and seeds. The application of these methods requires the extraction of an adequate amount of amplifiable DNA from the relevant matrix.

The DNA sequence amplified by the *P-nos* element-specific method can be detected in samples which contain DNA of the naturally occurring Ti-plasmid of *A. tumefaciens*. For this reason, it is necessary to confirm a positive screening result. Further analyses are required using construct-specific or event specific methods.

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

ISO 21570, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Quantitative nucleic acid based methods*

ISO 21571:2005, *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.