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

**Part 3:
Construct-specific real-time PCR
method for detection of P35S-pat-
sequence for screening genetically
modified organisms**

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

*Partie 3: Méthode PCR en temps réel spécifique de la construction
pour la détection de la séquence P35S-pat pour criblage des
organismes génétiquement modifiés*



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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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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 WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

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

ISO 21569 consists of the following parts, under the general title *Horizontal methods for molecular biomarker analysis — Methods of analysis for the detection of genetically modified organisms and derived products*:

- *Part 2: Construct-specific real-time PCR method for detection of event FP967 in linseed and linseed products* [Technical Specification]
- *Part 3: Construct-specific real-time PCR method for the detection of the P35S-pat-sequence for screening for compounds of genetically modified organisms* [Technical Specification]

ISO 21569:2005 is to be revised to become the future Part 1.

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

Part 3: Construct-specific real-time PCR method for detection of P35S-pat-sequence for screening genetically modified organisms

1 Scope

This Technical Specification describes a procedure for the detection of the DNA transition sequence between the 35S promoter (*P35S*) from *Cauliflower mosaic virus* and a modified phosphinothricin-acetyltransferase gene (*pat*) from *Streptomyces viridochromogenes*. The *P35S-pat* construct is frequently found in genetically modified plants with tolerance for phosphinothricin-containing herbicides. The *P35S-pat* construct specific method is based on a real-time PCR and can be used for qualitative and quantitative screening purposes. For identification and quantification of a specific event, a follow-up analysis has to be carried out.

This Technical Specification is applicable for the analysis of DNA extracted from foodstuffs. It may 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 quantity and quality of amplifiable DNA from the relevant matrix.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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 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 24276 apply.

4 Principle

DNA is extracted from the test portion applying a suitable method. The DNA analysis consists of two parts, namely,

- 1) verification of the amount and amplifiability of the extracted DNA, e.g. by means of a target taxon specific real-time PCR (see ISO 21570^[10]), and