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

Part 7: Real-time PCR methods for the detection of CaMV and Agrobacterium Ti-plasmid derived DNA sequences

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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 7: Real-time PCR based methods for the detection of CaMV and *Agrobacterium* Ti-plasmid derived DNA sequences

1 Scope

This document specifies a procedure for the detection of a DNA sequence of the open reading frame five (ORF V) from cauliflower mosaic virus (CaMV) and a procedure for the detection of the DNA sequence of the nopaline synthase (*nos*) gene from tumour-inducing (Ti) plasmids of phytopathogenic *Rhizobium radiobacter* (formerly named *Agrobacterium tumefaciens*). The procedures can be used in the context of screening for genetically modified crop/plants and their derived products to further clarify a positive PCR result for a specific promoter or terminator of CaMV (P-35S, T-35S), or both, and the *nos* gene (P-*nos*, T-*nos*), respectively.

The methods specified in this document will detect and identify naturally occurring CaMV or *Rhizobium radiobacter* (Ti plasmid) DNA, or both, if present in the sample in the absence of a genetically modified plant event containing the specified target sequences.

Both methods are based on the real-time polymerase chain reaction (PCR) and are applicable for the analysis of DNA extracted from foodstuffs and other products such as feedstuffs and seeds/grains. The application of the methods requires the extraction of an adequate amount of amplifiable DNA from the relevant matrix.

With appropriate calibration material, the CaMV ORF V or *nos* copy number, or both, can be estimated and compared, respectively, with the estimated copy number for the promoter (P-35S, P-*nos*) or the terminator (T-35S, T-*nos*) sequences, or both. Thereby, conclusions are possible about the presence of an unknown genetically modified organism (GMO) in addition to any detected CaMV DNA or *Rhizobium radiobacter* Ti plasmid DNA, or both, in a test sample.

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 21569:2005, *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*

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