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

Part 8: DNA extraction from alfalfa seeds and real-time PCR based detection methods for genetically modified alfalfa events J101, J163 and KK179

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

Partie 8: Extraction d'ADN à partir de graines de luzerne et méthodes de détection spécifiques à l'événement basées sur la PCR en temps réel pour les lignées de luzerne génétiquement modifiées J101, J163 et KK179

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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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 8: DNA extraction from alfalfa seeds and real-time PCR based detection methods for genetically modified alfalfa events J101, J163 and KK179

1 Scope

This document specifies procedures for DNA extraction from alfalfa (*Medicago sativa*) seeds and for the specific detection of the herbicide-tolerant alfalfa events J101 and J163 and the lignin-modified alfalfa event KK179 in crop/plant/seed/grain test samples.

The detection methods are based on real-time PCR and are targeting the DNA transition sequences between the alfalfa genome and the respective integrated gene construct. The methods can be applied for direct event-specific identification or as a follow-up analysis, if sequences encoding the promoter of the Figwort mosaic virus (*P-FMV*), the terminator of the nopaline synthase gene from *Rhizobium radiobacter* (*T-nos*), or the construct CTP2/CP4-EPSPS (herbicide tolerance) were detected by screening analyses of test samples.

In this document, the methods were validated using ground alfalfa seeds and DNA extracted thereof. The PCR methods are also applicable for the analysis of other matrices such as feed and foodstuffs. The application of these PCR methods 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 as described in this document or by applying another suitable method with comparable performance characteristics (see ISO 21571). The DNA analysis consists of two parts:

a) verification of the amount, quality and amplifiability of the extracted DNA, by means of the real-time PCR for the alfalfa-specific reference gene *acc1;*[1]

b) analysis using the real-time PCR detection methods specific for the alfalfa events J101, J163 and KK179, respectively.[2][3][4]

Detection of PCR products is done using specific hydrolysis probes labelled with fluorescent dyes (FAM as reporter dye and one or two quenchers) that bind the respective target sequences between the two primers (so-called "TaqMan chemistry"[5]).

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 Interlaboratory trial and control material

5.2.1 Ground alfalfa seeds, of a wild-type line and the events J101, J163 and KK179.[1)]

5.2.2 Plasmid DNAs, containing the target sequences of the J101, J163 and KK179 event-specific PCR methods.[2][3][4]

5.3 DNA extraction reagents and solutions

5.3.1 Hexadecyl-trimethyl-ammonium-bromide (CTAB) ($C_{19}H_{42}BrN$).

5.3.2 Glycogen, $\rho(CHCL_3)$ = 20 mg/l.

5.3.3 Potassium acetate solution, $c(CH_3COOK)$ = 5 mol/l (storage at –20 °C).

5.3.4 Chloroform, φ ($CHCl_3$) ≥ 99,5 %.

5.3.5 Hydrochloric acid, φ(HCl) = 37 %.

5.3.6 Isopropyl alcohol, $\varphi[CH_3CH(OH)CH_3]$ ≥ 99,5 %, (–20 °C; fresh aliquot).

5.3.7 Ethanol solution, $\varphi(C_2H_5OH)$ = 70 %, (fresh aliquot).

1) This product is supplied by Forage Genetics Intl., LLC, West Salem, Wisconsin, USA. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the supplier named.