TECHNICAL SPECIFICATION

ISO/TS 21569-6

First edition
2016-11-01

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

Part 6: Real-time PCR based screening methods for the detection of *cry1Ab/Ac* and *Pubi-cry* DNA sequences

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

Partie 6: Méthodes de dépistage PCR en temps réel pour la détection de séquences ADN cry1Ab/Ac *et* Pubi-cry

Reference number
ISO/TS 21569-6:2016(E)

COPYRIGHT PROTECTED DOCUMENT

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

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 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 6: Real-time PCR based screening methods for the detection of *cry1Ab/Ac* and *Pubi-cry* DNA sequences

1 Scope

This document specifies a procedure for the detection of a DNA sequence of the modified *cry1Ab/Ac* gene and a procedure for the detection of the DNA transition sequence between the maize ubiquitin promoter (*Pubi)* and the *cry1Ab/Ac* gene. The modified *cry1Ab/Ac* gene and the *Pubi-cry* construct are frequently found in genetically modified Bt plants. Both detection 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.

This document 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 these 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 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.

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

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

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

4 Principle

DNA is extracted from the test portion 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 target taxon specific real-time PCR (according to ISO 21570, see also Reference [1];

b) detection of the *cry1Ab/Ac* and/or the *Pubi-cry* DNA sequence in a real-time PCR, see References [2] and [3].

5 Reagents and materials

5.1 General

For the purpose of this document, only chemicals and water of recognized analytical grade, appropriate for molecular biology shall be used. Unless stated otherwise, solutions should be prepared by dissolving the corresponding reagents in water and be autoclaved. 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 polymerases which lead to equal or better results may also be used.

5.2.3 Oligonucleotides (see Tables 1 and 2).

Table 1 — Oligonucleotides for detection of *cry1Ab/Ac*

Name	DNA sequence of the oligonucleotide	Final concentration in the PCR
cry1Ab/Ac as target sequence[2]:		
Bt-F1(mod)	5'-gAg gAA ATg CgT ATT CAA TTC AAC-3'	400 nmol/l
Bt-R	5'-TTC Tgg ACT gCg AAC AAT gg-3'	400 nmol/l
Bt-P	5'-(FAM)-ACA TgA ACA gCg CCT TgA CCA CAg C-(NFQ)-3'[a]	100 nmol/l
[a] FAM: 6-Carboxyfluorescein, NFQ: non-fluorescent quencher (dark quencher).		

Table 2 — Oligonucleotides for detection of *Pubi-cry*

Name	DNA sequence of the oligonucleotide	Final concentration in the PCR
Pubi-cry as target sequence[2]:		
Pubi-F2	5'-ATT TgC TTg gTA CTg TTT CTT TTg TC-3'	300 nmol/l
Cry-rr-R	5'-TTg TTg TCC ATg gAT CCT CTA gAg T-3'	600 nmol/l
Pubi-T2	5'- (FAM)- ACC CTg TTg TTT ggT gTT ACT TCT gCA-(NFQ)-3'[a,b]	100 nmol/l
[a] FAM: 6-Carboxyfluorescein, NFQ: non-fluorescent quencher (dark quencher).		
[b] The Pubi-T2 probe is three bases shorter than the probe described in Reference [3] but identical specificity and sensitivity is achieved.		