SLOVENSKI STANDARD
SIST EN 15634-1:2009

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Živila - Odkrivanje prisotnosti alergenov v živilih z metodami molekularne biologije - 1. del: Splošne ugotovitve

Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations

Lebensmittel - Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 1: Allgemeine Betrachtungen

Produits alimentaires - Méthodes d'analyse de biologie moléculaire pour la détection des allergènes alimentaires - Partie 1 : Considérations générales

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Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations

Produits alimentaires - Détection des allergènes alimentaires par des méthodes d'analyse de biologie moléculaire - Partie 1: Considérations générales

Lebensmittel - Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 1: Allgemeine Betrachtungen

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: Avenue Marnix 17, B-1000 Brussels

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Foreword

This document (EN 15634-1:2009) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horitontal methods”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by July 2009, and conflicting national standards shall be withdrawn at the latest by July 2009.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

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Introduction

This European Standard describes the procedure to qualitatively detect and/or quantitate DNA as markers for potentially allergenic ingredients or constituents by analysing the nucleic acids extracted from the sample under study.

The qualitative detection of DNA targets is performed in order to get a yes or no answer to the question whether a certain DNA fragment is detected or not relative to appropriate controls and within the detection limits of the analytical method used and the test portion analysed.

The quantitative detection of DNA targets is performed to express the quantity of DNA targets, relative to the quantity of a specific reference, appropriate calibrants and controls and within the dynamic range of the analytical method used and the test portion analysed. Appropriate procedures for extraction of nucleic acids are included in each method.

The main focus of this European Standard will be on PCR based amplification methods. However, because of the rapid rate of technological change in this area, other amplification technologies and detection methods may be considered.

1 Scope

This European Standard provides the overall framework for detection of sequences corresponding to species containing allergens using the polymerase chain reaction (PCR). It relates to the requirements for the specific amplification of target nucleic acid sequences (DNA) and for the confirmation of the identity of the amplified nucleic acid sequence.

Guidelines, minimum requirements and performance criteria laid down in the European Standard are intended to ensure that comparable and reproducible results are obtained in different laboratories. This European Standard has been established for food matrices.

2 Normative references

The following referenced documents are indispensable for the application 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.

prEN 15842:2008, *Foodstuffs – Detection of food allergens – General considerations and validation of method*s

3 Terms and definitions

For the purposes of this document, the terms and definitions given in prEN 15842:2008 and the following apply.

3.1 Terms relative to extraction and purification of DNA

3.1.1
DNA extraction
separation of DNA from the other components in a test sample

[EN ISO 24276:2006] [1]

NOTE The factors of major importance for the isolated DNA are: a) purity, b) amount or concentration and c) quality (integrity).

3.1.2
DNA purification
method resulting in a higher purity of the extracted DNA

NOTE In this context, purity refers to the reduction of observable measurable effects of PCR inhibitors.

3.1.3
PCR quality
DNA template of sufficient quality to be amplified by PCR

3.2 Terms relative to amplification of DNA

3.2.1
species (class/order/family/genus) specific target sequence
sequence known to be specific for the species

3.2.2
identification of nucleic acid sequences
identification by comparison with a reference nucleic acid fragment/sequence

NOTE Identification is possible by e.g. positive hybridisation with probe, matching restriction digest profiles or matching nucleic acid sequences.

3.3 Definitions referring to controls

3.3.1
positive DNA target control
reference DNA or DNA extracted from a certified reference material or known positive samples representative of the sequence or target under study

NOTE The control is intended to demonstrate what the result of analyses of test sample containing the sequence under study will be.

3.3.2
negative DNA target control
reference DNA or DNA extracted from a certified negative (blank matrix) reference material or known negative sample not containing the sequence under study

NOTE The control is intended to demonstrate what the result of analyses of test samples not containing the sequence under study will be.

3.3.3
PCR inhibition control
control containing known amounts of positive template DNA added in the same amount as analyte DNA to the reaction

NOTE This control allows the determination of the presence of soluble PCR inhibitors, particularly necessary in case of negative amplification and of quantitative PCR.

3.3.4
amplification reagent control
control containing all the reagents, except extracted test sample template DNA

NOTE 1 Instead of the template DNA, a corresponding volume of nucleic acid free water is added to the reaction.

NOTE 2 The water used should be double distilled or equivalent, free from DNA and nucleases (molecular biology grade).

3.3.5
extraction blank control
control performing all steps of the extraction procedure, except addition of the test portion, e.g. by substitution of water for the test portion

NOTE 1 It is used to demonstrate the absence of contaminating nucleic acid during extraction.

NOTE 2 The water used should be double distilled or equivalent, free from DNA and nucleases (molecular biology grade).

3.3.6
positive extraction control
control sample meant to demonstrate that the nucleic acid extraction procedure has been performed in a way that will allow for extraction and subsequent amplification of the target nucleic acid, i.e. by using a sample material known to contain the target nucleic acid

NOTE Information about controls can be found in EN ISO 24276.

4 General laboratory requirements

4.1 General

A draft European Standard dealing with General considerations and validation criteria of methods was adopted as prEN 15842:2008.

4.2 Laboratory organisation

Compliance with applicable requirements with respect to safety regulations and manufacturer's safety recommendation shall be followed.

Accidental contamination of DNA can originate from dust or spreading aerosols. As a consequence, the organisation of the work area in the laboratory is logically based on:

— the systemic containment of the methodological steps involved in the production of the results, and

— a forward flow principle for sample handling.

A minimum of three separately designated work areas with their own apparatus is required:

a) a work area for extraction of the nucleic acid from the test portion (sample);

b) a work area dedicated to the set up of PCR/amplification reactions; and

c) a work area dedicated for subsequent processing including analysis and characterisation of the amplified DNA segments.

If dust particle producing grinding techniques are used, this has to be carried out in a separate work area.

Physical separation through the use of different rooms is the most effective and preferable way of ensuring separated work areas, but other physical or biochemical methods may be used as a protection against contamination provided their effectiveness is comparable.

Staff shall wear different sets of lab coats at each dedicated work area. They shall also wear disposable gloves. Gloves and lab coats should be changed at appropriated frequencies.

4.3 Apparatus and equipment

The laboratory should use properly maintained equipment suitable for the method employed, e.g. according to the requirements outlined by EN ISO/IEC 17025 [2]. In addition to standard laboratory equipment, additional apparatus are described in the specific methods.

Apparatus and equipment shall be maintained according to manufacturer's instructions. Calibration systems shall be available and calibration routinely performed for equipment which may impact the data produced, according to laboratory quality assurance programs.

4.4 Material and reagents

For the analysis, unless otherwise stated, use only analytically pure reagents suitable for molecular biology, free from DNA and DNAses. Reagents and solutions should be stored at room temperature, unless otherwise specified. PCR reagents should be stored in small aliquots to minimize the risk of contamination. The water used shall be double distilled or equivalent, free from DNA and nucleases (molecular biology grade). Solutions are prepared by dissolving the appropriate reagents in water and autoclaved unless specified differently. Ultra-filtration devices can be used, when autoclaving is not possible.