SLOVENSKI STANDARD
SIST EN 15634-1:2019

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Nadomešča:
SIST EN 15634-1:2009

Živila - Odkrivanje prisotnosti alergenov v živilih z molekularno biološkimi metodami - 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 - Détection des allergènes alimentaires par des méthodes d'analyse de biologie moléculaire - Partie 1: Considérations générales

Ta slovenski standard je istoveten z: **EN 15634-1:2019**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology
67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

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EUROPEAN STANDARD

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English Version

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

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

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European foreword

This document (EN 15634-1:2019) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal 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of an identical text or by endorsement, at the latest by April 2020, and conflicting national standards shall be withdrawn at the latest by April 2020.

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

This document supersedes EN 15634-1:2009.

Significant technical changes between this standard and EN 15634-1:2009 are as follows:

a) updated terms and definitions (3);

b) requirements regarding the preparation of samples changed (6.1);

c) clause 6.3 on DNA quantitation changed;

d) clause 7.2.1 on primer design changed;

e) requirements regarding quantitation of PCR products (8.2) changed;

f) clause on "Quality assurance requirements" deleted;

g) the test report should comply with EN ISO/IEC 17025;

h) updated bibliography.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

This document describes the procedure to qualitatively detect and/or quantitate DNA fragments 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 sequence 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 document 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.

For the use of this document the term:

— ‘shall’ indicates a requirement;

— ‘should’ indicates a recommendation;

— ‘may’ indicates a permission; and

— ‘can’ indicates a possibility and/or a capability.

1 Scope

This document 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 European Standards are intended to ensure that comparable and reproducible results are obtained in different laboratories. This document has been established for food matrices.

This document is intended to be used in addition to EN 15842.

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.

EN 15842, *Foodstuffs — Detection of food allergens — General considerations and validation of methods*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 15842 and the following 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 https://www.iso.org/obp

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

Note 1 to entry: The factors of major importance for the isolated DNA are:

a) purity,

b) amount or concentration and

c) quality (integrity).

[SOURCE: EN ISO 24276:2006, 3.2.1, modified — note was added]

3.1.2
DNA purification
method resulting in a DNA intended to reduce observable measurable effects of PCR inhibitors

Note 1 to entry: In this context, purity refers to the reduction of observable measurable effects of PCR inhibitors.

3.1.3
PCR quality DNA
DNA template of sufficient length, chemical purity, and structural integrity to be amplified by PCR

3.2 Terms relative to amplification of DNA

3.2.1
species specific target sequence
sequence known to be specific for the species or higher taxa

3.2.2
identification of amplified nucleic acid sequences
proof of identity of amplified nucleic acid sequences (amplicons) by comparison with a reference nucleic acid fragment pattern or sequence

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 1 to entry: The control is intended to demonstrate the result of analyses of test sample containing the sequence under study.

[SOURCE: EN ISO 24276:2006, 3.4.1, modified — replaced organism by target, note was changed]

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 1 to entry The control is intended to demonstrate the result of analyses of test samples not containing the sequence under study.

[SOURCE: EN ISO 24276:2006, 3.4.2, modified — note was changed]

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

Note 1 to entry: This control allows the detection of the presence of soluble PCR inhibitors, particularly necessary in case of non-specific or none amplification and of quantitative PCR.

3.3.4
amplification reagent control
no template control
control containing all the reagents, except extracted test sample template DNA

Note 1 to entry: Instead of the template DNA, a corresponding volume of nucleic acid free water or buffer is added to the reaction.

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 to entry: It is used to detect possible contaminating nucleic acid in a buffer or chemicals used during extraction.

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

Note 1 to entry: The DNA amount is usually e.g. tenfold over the LOD of the method to ensure amplification.

4 General requirements for laboratories

4.1 General

In addition to EN ISO/IEC 17025, also EN 15842 dealing with general considerations and validation criteria of methods exists.

4.2 Laboratory organization

Compliance with applicable requirements with respect to safety regulations should be followed and manufacturer's safety recommendation should be taken into account.

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

— the systemic containment of the methodological steps involved in the analysis, 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 setup of PCR/amplification reactions; and

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

If dust particle producing grinding techniques are used, this shall 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 should preferably 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.