Foodstuffs - Detection of food allergens by molecular biological methods - Part 4: Peanut (Arachis hypogaea) - Qualitative detection of a specific DNA sequence in chocolate by real-time PCR

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

See Eesti standard EVS-EN 15634-4:2023 sisaldab Euroopa standardi EN 15634-4:2023 ingliskeelset teksti.	This Estonian standard EVS-EN 15634-4:2023 consists of the English text of the European standard EN 15634-4:2023.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas.	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation and Accreditation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 22.02.2023.	Date of Availability of the European standard is 22.02.2023.
Standard on kättesaadav Eesti Standardimis-ja Akrediteerimiskeskusest.	The standard is available from the Estonian Centre for Standardisation and Accreditation.

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

ICS 07.100.30, 67.190

EUROPEAN STANDARD

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English Version

Foodstuffs - Detection of food allergens by molecular biological methods - Part 4: Peanut (*Arachis hypogaea*) - Qualitative detection of a specific DNA sequence in chocolate by real-time PCR

Produits alimentaires - Détection des allergènes alimentaires par des méthodes d'analyse de biologie moléculaire - Partie 4 : Arachide (*Arachis hypogaea*) - Détection qualitative d'une séquence d'ADN spécifique dans du chocolat, par PCR en temps réel

Lebensmittel - Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 4: Erdnuss (*Arachis hypogaea*) - Qualitativer Nachweis einer spezifischen DNA-Sequenz in Schokolade mittels Real-time PCR

This European Standard was approved by CEN on 16 January 2023.

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European foreword

This document (EN 15634-4:2023) 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of an identical text or by endorsement, at the latest by August 2023, and conflicting national standards shall be withdrawn at the latest by August 2023.

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 CEN/TS 15634-4:2016.

In comparison with CEN/TS 15634-4:2016, the following technical modifications have been made:

a) the document was converted from a Technical Specification into a European standard;

b) normative references and terms and definitions clause added;

c) PCR controls moved from Clause 3 “Reagents” to Clause 7 “Procedure”;

d) new subclause 7.4.9 “Accept/Reject criteria” added;

e) restructured clauses in alignment with EN 15634-2:2019.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

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, Türkiye and the United Kingdom.

Introduction

For the use of this document the term:

— ‘shall’ indicates a requirement;

— ‘should’ indicates a recommendation;

— ‘may’ indicates a permission;

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

1 Scope

This document specifies a method for the detection of peanut (*Arachis hypogaea*) in chocolate.

Real-time PCR (Polymerase Chain Reaction) detection of peanut is based on an 86 bp (base pair) sequence from the Ara h 2 gene of peanut.

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 15634-1:2019, *Foodstuffs - Detection of food allergens by molecular biological methods - Part 1: General considerations*

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 EN 15634-1 apply.

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

- IEC Electropedia: available at https://www.electropedia.org/
- ISO Online browsing platform: available at https://www.iso.org/obp/ui

4 Principle

Total DNA from chocolate is extracted from the sample using a cetyltrimethylammoniumbromide (CTAB) method. Potential PCR inhibitors are removed from the DNA extracted by purification with solid phase columns and the DNA content is measured by photospectrometry. Real-time PCR is used to detect a peanut specific DNA sequence. The real-time PCR method involves a fluorescence detection with a sequence specific hydrolysis probe [1], [2].

5 Reagents

5.1 General

The following general conditions for analysis should be followed, unless specified differently. Use only analytical grade reagents suitable for molecular biology. All water shall be free from DNA and nucleases, e.g. double distilled or equivalent (molecular grade). Solutions shall be prepared by dissolving the appropriate reagents in water and autoclaving, unless specified differently.

5.2 Extraction reagents

5.2.1 Chloroform.

5.2.2 Ethanol, volume fraction φ = 70 %.

5.2.3 Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA).

5.2.4 Cetyltrimethylammoniumbromide (CTAB).

5.2.5 Hydrochloric acid, mass fraction w = 37 %.