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Molecular biomarker analysis — Isothermal polymerase chain reaction (isoPCR) methods —

Part 1: General requirements

Analyse de biomarqueurs moléculaires — Méthodes de réaction de polymérisation en chaîne isotherme (isoPCR) —

Partie 1: Exigences générales

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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 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).

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For an explanation of the voluntary nature of standards, 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 www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

A list of all parts in the ISO 22942 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.

Introduction

Isothermal nucleic acid amplification describes methods that use constant temperature polymerase-catalysed reactions to amplify a nucleic acid target sequence[1][2][3][4][5][6]. In contrast to thermal-cycler based polymerase chain reactions, isothermal nucleic acid amplification does not require variable temperature cycling for denaturation, annealing, and polymerization although, in some cases, primer binding requires a single high temperature denaturation and an annealing step. Isothermal amplification methods can be described by the term "isothermal PCR (isoPCR)".

Naturally, living organisms isothermally replicate DNA during cell division and transcribe RNA to produce structural, and regulatory components. IsoPCR leverages both natural and synthetic isothermal enzymatic processes. The enzymes include DNA and RNA polymerase, helicase, recombinase, exonuclease and nickase. Because isoPCR does not require variable temperature cycling for denaturation, polymerization and annealing there is no need for precision thermal cycling instruments. Reactions are run at a single temperature, except in cases where a nickase or displacing enzyme is not present in the reaction and an initial denaturation is required. In addition, various non-enzymatic nucleic acid binding proteins can be necessary. IsoPCR amplification in many applications can be performed on cell lysates without nucleic acid extraction. Some examples of amplification strategies are loop-mediated isothermal amplification (LAMP)[7], rolling circle amplification (RCA)[8], helicase dependent amplification (HDA)[9], recombinase polymerase amplification (RPA)[10], strand displacement amplification (SDA)[11], nucleic acid sequence-based amplification (NASBA)[12] and Cas9 nickase-based amplification reaction (Cas9nAR)[13]. The LAMP, RCA, HDA, RPA, SDA and NASBA strategies can incorporate both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) into amplified nucleic acids. Cas9nAR can only use DNA as the starting template for amplification.

IsoPCR methods can be used for amplification, detection, identification, quantification, and analysis of specific low concentration nucleic acids in food and food products. These methods can, in most cases, amplify nucleic acids from un-purified nucleotide extracts. Detection of the target sequence is achieved through real-time or end-point techniques using one of several different amplification strategies and detection chemistries. Detection chemistries include turbidimetry, chromatography, gel electrophoresis and fluorescence, and can, in some applications, be achieved in a closed lateral flow device system.

Key features of isoPCR methods are constant temperature nucleic acid amplification, use of crude extracts, simple detection methods, and short reaction times without the need for precision thermal cycling instruments.

Because isoPCR methods are gaining in popularity and applicability, standardization of the acceptance criteria for these methods in food products is important.

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Part 1: General requirements

1 Scope

This document specifies general criteria for development, validation and use of nucleic acid analytical methods based on the isothermal polymerase chain reaction (isoPCR). It provides additional information and guidance for specific isoPCR technologies.

This document is applicable to food, feed, plant matrices and their propagules, plant pathogens, and animals in which amplification of a specific biomolecular target sequence is required.

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/TS 16393, *Molecular biomarker analysis — Determination of the performance characteristics of qualitative measurement methods and validation of methods*

ISO 16577, *Molecular biomarker analysis — Vocabulary for molecular biomarker analytical methods in agriculture and food production*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 16577 and the following 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/

3.1
extraction blank control
negative control reaction generated by performing all required steps in an extraction procedure except for the addition of the test portion

EXAMPLE By substitution of water for the test portion.

Note 1 to entry: This control is used to demonstrate the absence of contamination during extraction.

3.2
extraction control
positive control reaction generated by performing all required steps in an extraction procedure except with a known test portion containing a known amount of target nucleic acid or tissue

Note 1 to entry: This control is used to demonstrate the performance of the extraction process.