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**Meat and meat products —  
Determination of L-(+)-glutamic acid  
content — Reference method**

*Viande et produits à base de viande — Détermination de la teneur en  
acide L-(+)-glutamique — Méthode de référence*



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## 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](http://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](http://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 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](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 6, *Meat, poultry, fish, eggs and their products*.

This third edition cancels and replaces the second edition (ISO 4134:1999), which has been technically revised. The main changes compared with the previous edition are as follows:

- a new test method, the light absorption microplate reader method, has been added;
- the order of the clauses has been rearranged;
- the Scope ([Clause 1](#)) has been revised to specify free L-(+)-glutamic acid in meat and meat products;
- the Normative references ([Clause 2](#)) have been updated;
- the Terms and definitions ([Clause 3](#)) have been modified by adding the term “free L-(+)-glutamic acid”;
- in [Clause 4](#), the description of “extraction of L-(+)-glutamic acid of test portion” has been modified and the detection wavelength has been changed from “492 nm” to “490 nm”;
- in [7.1](#), the identification of enzyme activity units for diaphorase and glutamate dehydrogenase has been supplemented; the concentration of KOH, NAD has been modified; the NAD and diaphorase have been mixed into a solution; and the buffer, NAD and enzymes have been labelled with R1, R2, and R3;
- the apparatus list ([7.2](#)) has been updated;
- in [7.3](#), the procedure of the test method of spectrophotometer has been modified by halving the sample mass and solution volume;
- in [7.3.4](#), the method of judging the absorbance of the reaction end point has been modified and, as a result, the previous Annex B “Example of plotting and extrapolation of absorbance values” has been deleted;

- in 8.4, the formula and symbol description of spectrophotometer has been modified;
- the previous Annex C “Derivation of equation for calculation of L-(+)-glutamic acid content” has been deleted;
- the Bibliography has been updated.

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](http://www.iso.org/members.html).



# Meat and meat products — Determination of L-(+)-glutamic acid content — Reference method

## 1 Scope

This document specifies the spectrophotometer method and the light absorption microplate reader method for the determination of the free L-(+)-glutamic acid content of meat and meat products.

This document is applicable to meat and meat products, including livestock and poultry products.

## 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 648, *Laboratory glassware — Single-volume pipettes*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

ISO 1442, *Meat and meat products — Determination of moisture content (Reference method)*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 8655-2, *Piston-operated volumetric apparatus — Part 2: piston pipettes*

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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

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

### 3.1

#### free L-(+)-glutamic acid

L-(+)-glutamic acid and glutamate existing in meat and meat products in the form of free state

## 4 Principle

The free L-(+)-glutamic acid present in a test portion is extracted with perchloric acid solution. The extract is centrifuged, decanted and filtered, and diluted to appropriate concentration with water, and the pH is adjusted to 10. Nicotinamide adenine dinucleotide (NAD) is reduced by the L-(+)-glutamic acid in the presence of glutamate dehydrogenase, see [Formula \(1\)](#). The resultant reduced nicotinamide adenine dinucleotide (NADH) reacts with idonitrotetrazolium chloride in the presence of diaphorase, see [Formula \(2\)](#). The resulting formazane is measured at a wavelength of 490 nm and the free L-(+)-glutamic acid content of the test sample is calculated.

