

INTERNATIONAL
STANDARD

ISO
11816-2

IDF
155-2

Second edition
2016-08-15

**Milk and milk products —
Determination of alkaline
phosphatase activity —**

Part 2:
Fluorimetric method for cheese

*Lait et produits laitiers — Détermination de l'activité de la
phosphatase alcaline —*

Partie 2: Méthode fluorimétrique pour le fromage



Reference numbers
ISO 11816-2:2016(E)
IDF 155-2:2016(E)

© ISO and IDF 2016

This document is a preview generated by EMS



COPYRIGHT PROTECTED DOCUMENT

© ISO and IDF 2016, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

International Dairy Federation
Silver Building • Bd Auguste Reyers 70/B • B-1030 Brussels
Tel. + 32 2 325 67 40
Fax + 32 2 325 67 41
info@fil-idf.org
www.fil-idf.org

Contents

	Page
Forewords	iv
1 Scope	1
2 Normative reference	1
3 Terms and definitions	1
4 Principle	1
5 Reagents	2
6 Apparatus	3
7 Sampling	4
8 Preparation of test sample	4
9 Procedure	4
9.1 Verification of instrument performance	4
9.1.1 General	4
9.1.2 Daily instrument tests	5
9.1.3 Controls	5
9.2 Reagent controls to test the suitability of ready to use working substrate (5.3)	5
9.3 Calibration	6
9.4 Determination	6
9.5 Test sample related controls	7
9.5.1 Recommended negative and positive control tests	7
9.5.2 Interfering substance test	8
9.5.3 Heat-stable microbial alkaline phosphatase control test	8
10 Calculation and expression of results	8
10.1 Calibration ratio	8
10.2 Calculation	9
10.2.1 Supernatant	9
10.2.2 Cheese	9
10.3 Expression of results	10
11 Precision	10
11.1 Interlaboratory test	10
11.2 Repeatability	10
11.3 Reproducibility	10
12 Test report	10
Annex A (informative) Interlaboratory trial	11
Annex B (informative) Examples of preparation of a test sample	13
Bibliography	15

Forewords

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).

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).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products* and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

This second edition of ISO 11816-2|IDF 155-2 cancels and replaces the first edition (ISO 11816-2|IDF 155-2:2003), which has been technically revised.

ISO 11816|IDF 155 consists of the following parts, under the general title *Milk and milk products — Determination of alkaline phosphatase activity*:

- *Part 1: Fluorimetric method for milk and milk-based drinks*
- *Part 2: Fluorimetric method for cheese*

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF 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).

Any trade name used in this document is information given for the convenience of users and does not constitute and endorsement

ISO 11816|IDF 155 was prepared by the IDF Standing Committee on *Analytical Methods for Processing Aids and Indicators* and ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*.

The work was carried out by the IDF/ISO Project Group on *Alkaline phosphatase activity in cheese (P06)*, of the Standing Committee on *Analytical Methods for Processing Aids and Indicators*, under the aegis of its project leader Mrs. M. Nicolas (FR).

This ISO/IDF International Standard cancels and replaces ISO 11816-2|IDF 155-2:2003, which has been technically revised.

ISO 11816|IDF 155 consists of the following parts, under the general title *Milk and milk products — Determination of alkaline phosphatase activity*:

- *Part 1: Fluorimetric method for milk and milk-based drinks*
- *Part 2: Fluorimetric method for cheese*

Milk and milk products — Determination of alkaline phosphatase activity —

Part 2: Fluorimetric method for cheese

1 Scope

This part of ISO 11816|IDF 155 specifies a fluorimetric method for the determination of alkaline phosphatase (ALP, EC 3.1.3.1) activity in cheese.

This method is applicable to soft cheeses, semi-hard and hard cheeses provided that the mould is only on the surface of the cheese and not also in the inner part (e.g. blue veined cheeses). For large hard cheeses, specific conditions of sampling apply (see [Clause 7](#)).

The instrument can read activities in the supernatant up to 7 000 milliunits per litre (mU/l).

2 Normative reference

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

3 Terms and definitions

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

3.1

alkaline phosphatase activity

ALP activity

activity of the alkaline phosphatase present in the product, determined by the specified procedure

Note 1 to entry: The alkaline phosphatase activity is expressed as milliunits of enzyme activity per gram of sample (mU/g).

3.2

unit of alkaline phosphatase activity

amount of alkaline phosphatase enzyme that catalyses the transformation of 1 μmol of substrate per minute

4 Principle

The alkaline phosphatase activity of the sample is measured by a continuous fluorimetric direct kinetic assay. A non-fluorescent aromatic monophosphoric ester substrate, 2'-[2-benzothiazolyl]-6'-hydroxybenzothiazole phosphate, in the presence of any alkaline phosphatase derived from the sample, undergoes hydrolysis of its phosphate radical, producing a highly fluorescent product. Fluorimetric