



BSI Standards Publication

Milk and milk products — Determination of alkaline phosphatase activity — Fluorimetric microplate method

National foreword

This Published Document is the UK implementation of ISO/TS 4985:2023.

The UK participation in its preparation was entrusted to Technical Committee AW/5, Chemical analysis of milk and milk products.

A list of organizations represented on this committee can be obtained on request to its committee manager.

Contractual and legal considerations

This publication has been prepared in good faith, however no representation, warranty, assurance or undertaking (express or implied) is or will be made, and no responsibility or liability is or will be accepted by BSI in relation to the adequacy, accuracy, completeness or reasonableness of this publication. All and any such responsibility and liability is expressly disclaimed to the full extent permitted by the law.

This publication is provided as is, and is to be used at the recipient's own risk.

The recipient is advised to consider seeking professional guidance with respect to its use of this publication.

This publication is not intended to constitute a contract. Users are responsible for its correct application.

This publication is not to be regarded as a British Standard.

© The British Standards Institution 2023
Published by BSI Standards Limited 2023

ISBN 978 0 539 24427 4

ICS 67.100.10

Compliance with a Published Document cannot confer immunity from legal obligations.

This Published Document was published under the authority of the Standards Policy and Strategy Committee on 31 August 2023.

Amendments/corrigenda issued since publication

Date	Text affected

TECHNICAL
SPECIFICATION

ISO/TS
4985

IDF/RM 255

First edition
2023-07

**Milk and milk products —
Determination of alkaline
phosphatase activity — Fluorimetric
microplate method**



Reference numbers
ISO/TS 4985:2023(E)
IDF/RM 255:2023(E)

Documents
Document Sharing Hub
© ISO and IDF 2023



COPYRIGHT PROTECTED DOCUMENT

© ISO and IDF 2023

All rights reserved. Unless otherwise specified, or required in the context of its implementation, 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
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11

Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

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

Contents

Page

Forewords	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	1
5 Reagents	2
6 Apparatus and materials	3
7 Sampling	4
8 Preparation	4
8.1 Preparation of alkaline phosphatase-free sample	4
8.1.1 General	4
8.1.2 Alkaline phosphatase-free milk	4
8.1.3 Alkaline phosphatase-free cheese	4
8.2 Preparation of test sample	5
8.2.1 Milk samples	5
8.2.2 Cheese samples	5
9 Procedure	5
9.1 Instrument	5
9.1.1 Instrument settings	5
9.1.2 Test the linearity range of the instrument	6
9.1.3 Quality control	6
9.2 Calibration	6
9.2.1 General	6
9.2.2 Preparation of the working standard solutions	6
9.2.3 Preparation of the calibration curve	7
9.3 Determination	7
9.3.1 Milk samples	7
9.3.2 Cheese samples	8
9.3.3 Plate layout and fluorescence measurement	9
9.4 Test sample-related controls	9
9.4.1 Recommended negative and positive controls	9
9.4.2 Interfering substance test	10
9.4.3 Heat-stable microbial alkaline phosphatase control test	10
10 Calculation and expression of results	10
10.1 Calibration curve	10
10.2 Calculation of ALP activity of unknown samples	11
10.2.1 Milk samples	11
10.2.2 Cheese samples	12
10.2.3 Calculation example	13
10.3 Expression of results	14
11 Precision	14
11.1 Interlaboratory test	14
11.2 Repeatability	14
11.3 Reproducibility	14
12 Test report	15
Annex A (informative) Precision data	16
Annex B (informative) Method comparison	17
Annex C (informative) Examples of preparation of test sample	19

Bibliography	21
---------------------------	-----------

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 document 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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

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.

This document was prepared by Technical Committee 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.

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.

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 an endorsement.

This document 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*. It is being published jointly by ISO and IDF.

The work was carried out by the IDF/ISO Action Team on P20 of the *Standing Committee on Analytical Methods for Processing Aids and Indicators* under the aegis of its project leader Dr C. Egger (CH).

Milk and milk products — Determination of alkaline phosphatase activity — Fluorimetric microplate method

1 Scope

This document specifies a fluorimetric microplate method for the determination of alkaline phosphatase (ALP, EC 3.1.3.1)^[5] activity in raw and heat-treated whole milk, semi-skimmed milk, skimmed milk, cream, flavoured milks and cheeses.

This method is applicable to milk and milk-based drinks from cows, sheep and goats. Although the method was not tested in milk from other species, it can also be applicable to milk from other species with a similar composition to cow, sheep or goat milk, such as milk from buffalo and camelids. It is also applicable to milk powder after reconstitution and soft, 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](#)).

NOTE This method was adapted from Reference [\[6\]](#).

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

alkaline phosphatase activity

ALP activity

amount of enzyme that is capable of catalysing the transformation of 1 μmol of substrate per minute under the conditions of the specified procedure

Note 1 to entry: The ALP activity in milk is expressed as milliunits of enzyme activity per litre of sample (mU/l) and as milliunits of enzyme activity per gram of sample (mU/g) in the case of cheese.

4 Principle

The ALP activity of the sample is measured by a continuous fluorimetric direct kinetic assay. A non-fluorescent aromatic monophosphoric substrate, 4-methylumbelliferone phosphate (4-MUP), in the presence of any ALP derived from the sample, undergoes hydrolysis of its phosphate, producing the highly fluorescent product 4-methylumbelliferone (4-MU). Fluorimetric measurement of ALP activity is measured at 37 °C, over a 15 min period.

The measured fluorescence is proportional to the concentration of the emitted fluorescent product and is used to calculate the enzyme activity.