



SLOVENSKI STANDARD SIST EN ISO 11816-1:2001

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Milk and milk products - Determination of alkaline phosphatase activity using a fluorimetric method - Part 1: Milk and milk-based drinks (ISO 11816-1:1997)

Milch und Milchprodukte - Bestimmung der Aktivität der alkalischen Phosphatase mit einem fluorimetrischen Verfahren - Teil 1: Milch und flüssige Milchprodukte (ISO 11816-1:1997)

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Lait et produits laitiers - Détermination de l'activité de la phosphatase alcaline a l'aide de la méthode fluorimétrique - Partie 1: Lait et boissons a base de lait (ISO 11816-1:1997)

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Ta slovenski standard je istoveten z: EN ISO 11816-1:2000

ICS:

67.100.10 T | ^ \ [Á Á | ^ á ^ | æ ä | ^ } ä Milk and processed milk products
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EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN ISO 11816-1

March 2000

ICS 67.100.10

English version

Milk and milk products - Determination of alkaline phosphatase activity using a fluorimetric method - Part 1: Milk and milk-based drinks (ISO 11816-1:1997)

Lait et produits laitiers - Détermination de l'activité de la phosphatase alcaline à l'aide de la méthode fluorimétrique - Partie 1: Lait et boissons à base de lait (ISO 11816-1:1997)

Milch und Milchprodukte - Bestimmung der Aktivität der alkalischen Phosphatase mit einem fluorimetrischen Verfahren - Teil 1: Milch und flüssige Milchprodukte (ISO 11816-1:1997)

This European Standard was approved by CEN on 14 February 2000.

CEN members are bound to comply with the CEN/GENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Central Secretariat: rue de Stassart, 36 B-1050 Brussels

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Foreword

The text of the International Standard from Technical Committee ISO/TC 34/SC 5 "Milk and milk products" of the International Organization for Standardization (ISO) has been taken over as an European Standard by Technical Committee CEN/TC 302 "Milk and milk products - Methods of sampling and analysis", the secretariat of which is held by NNI.

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 September 2000, and conflicting national standards shall be withdrawn at the latest by September 2000.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

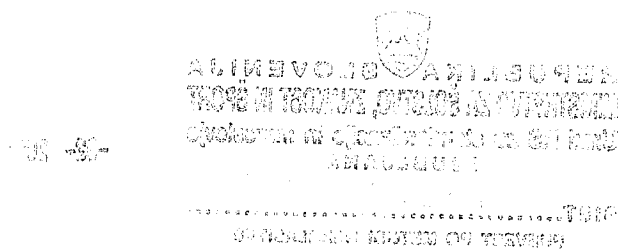
Endorsement notice

The text of the International Standard ISO 11816-1:1997 has been approved by CEN as a European Standard without any modification.

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INTERNATIONAL STANDARD

ISO 11816-1

First edition
1997-02-15

Milk and milk products — Determination of alkaline phosphatase activity using a fluorimetric method —

Part 1: Milk and milk-based drinks

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*Lait et produits laitiers — Détermination de l'activité de la phosphatase
alcaline à l'aide de la méthode fluorimétrique —*

Partie 1: Lait et boissons à base de lait

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Reference number
ISO 11816-1:1997(E)

ISO 11816-1:1997(E)

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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 11816-1 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with the International Dairy Federation (IDF) and AOAC INTERNATIONAL, and will also be published by these organizations.

ISO 11816 consists of the following parts, under the general title *Milk and milk products — Determination of alkaline phosphatase activity using a fluorimetric method*:

- Part 1: *Milk and milk-based drinks*
- Part 2: *Cheeses*

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Annex A of this part of ISO 11816 is for information only.

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Milk and milk products — Determination of alkaline phosphatase activity using a fluorimetric method —

Part 1:

Milk and milk-based drinks

1 Scope

This part of ISO 11816 specifies a fluorimetric method for the determination of alkaline phosphatase activity in pasteurized whole milk, semi-skimmed milk, skimmed milk and flavoured milks.

The method is also suitable for the determination of high alkaline phosphatase activity in raw milk and heat-treated milk having activities of more than 7 000 milliunits per litre.

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2 Definitions

For the purposes of this part of ISO 11816, the following definitions apply.

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2.1 alkaline phosphatase activity (APL): Activity of the alkaline phosphatase present in a product, determined in accordance with the procedure specified in this part of ISO 11816. It is expressed as milliunits per litre.

NOTE 1 See reference [1] in annex A.

2.2 unit of alkaline phosphatase activity: Amount of alkaline phosphatase enzyme that catalyses the transformation of 1 μmol of substrate per minute per litre of sample.

3 Principle

The alkaline phosphatase activity of the sample is measured by a continuous fluorimetric direct kinetic assay. A non-fluorescent aromatic monophosphoric ester substrate, in the presence of any alkaline phosphatase derived from the sample, undergoes hydrolysis of its phosphate radical, producing a highly fluorescent product. Fluorimetric measurement of alkaline phosphatase (ALP) activity is measured at 38 °C over a 3-min period.

NOTE 2 Although this is a 3-min test, the first minute is an equilibration period to ensure that the sample is at 38 °C. Measurements of activity are actually made from the beginning of the second minute to the end of the third minute (i.e. over a 2-min period).

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

4.1 Substrate, for example Fluorophos substrate¹⁾, crystallized.

NOTE 3 Fluorophos substrate is a water-soluble, non-fluorescent aromatic monophosphoric ester substrate, which is stable for 1 year when crystallized and stored in glass vials at 4 °C.

4.2 Substrate diluent¹⁾: diethanolamine (DEA) buffer (pH 10,0), 2,4 mol/l solution.

NOTE 4 The buffer solution is stable for 1 year at 4 °C.

4.3 Working substrate¹⁾

Add a volume of the substrate diluent (4.2) to the substrate (4.1) to give a concentration of 1,044 mmol/l and mix well by inversion. Use amber glass to protect against light.

If using the Fluorophos Test System¹⁾, add the content of 1 vial of the substrate diluent (4.2) to one vial of the substrate (4.1) and mix well by inversion.

NOTE 5 This solution is stable when stored in the dark for 8 weeks at 4 °C and 8 h at 38 °C. It is sufficient for 115 tests.

4.4 Working calibrators¹⁾, for example Fluoroyellow in DEA buffer.

4.4.1 Calibrator solution A, containing 0 µmol/l of Fluoroyellow.

4.4.2 Calibrator solution B, containing $17,24 \times 10^{-3}$ µmol/l of Fluoroyellow.

4.4.3 Calibrator solution C, containing $34,48 \times 10^{-3}$ µmol/l of Fluoroyellow.

NOTES

6 Calibrator solutions are stable for 1 year when stored at 4 °C.

7 The volumes of reagents supplied for the Fluorophos Test System may be changed by the manufacturer. The user should refer to the manufacturer's instructions for preparing the reagents if the volumes supplied are different from those specified in 4.1 to 4.4.

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 Filter fluorimeter¹⁾, with thermostatted cuvette holder maintained at $38 \text{ °C} \pm 1 \text{ °C}$, and right-angle optics, allowing excitation at a wavelength of 440 nm and emission at 560 nm.

5.2 Cuvettes, disposable, non-fluorescent glass, of diameter 12 mm and length 75 mm.

5.3 Fixed-volume dispenser, to dispense 2,0 ml.

5.4 Positive-displacement pipette, of capacity 0,075 ml.

5.5 Pipettes, of capacities 1 ml and 2 ml.

¹⁾ The reagents specified in 4.1 to 4.4 and the apparatus specified in 5.1 to 5.6 (except 5.5) are available as the Fluorophos Test System from Advanced Instruments Inc., Two Technology Way, Norwood, MA 02062, USA. Fluorophos and Fluoroyellow are registered trademarks of Advanced Instruments Inc. and are examples of suitable products available commercially. This information is given for the convenience of users of this part of ISO 11816 and does not constitute an endorsement by ISO of these products.

5.6 Incubator block, suitable to hold the cuvettes and capable of being maintained at a temperature of $38\text{ °C} \pm 1\text{ °C}$.

5.7 Parafilm or other suitable laboratory grade film.

5.8 Vortex shaker

5.9 Water bath, capable of being maintained at $95\text{ °C} \pm 1\text{ °C}$.

5.10 One-mark volumetric flasks, of capacity 100 ml.

6 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this part of ISO 11816. A recommended sampling method is given in ISO 707 [2].

7 Preparation of test sample

Carefully mix the laboratory sample. It is usually not necessary to prewarm the test sample.

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8 Procedure

8.1 Test portion

8.1.1 Pasteurized samples

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Use pasteurized test samples as delivered, in amounts as required.

8.1.2 Raw milk and heat-treated milk

Pipette (5.5) 1 ml of the test sample of raw milk into a volumetric flask (5.10). Dilute to 100 ml with phosphatase-free milk (8.4.1). Mix carefully. Dilute test samples of heat-treated milk with phosphatase-free milk (8.4.1) so as to obtain an alkaline phosphatase activity in the test portion of less than 7 000 milliunits per litre (mU/L).

8.2 Calibration

Establish a calibration curve for each type of product to be tested. Calibration curves are usually stable but the instrument shall be recalibrated every 2 to 3 months. Check the instrument if there are changes in the calibration curve.

Using the pipette (5.5), transfer 2,0 ml of the calibrator solutions A, B and C (4.4.1, 4.4.2 and 4.4.3 respectively), each in duplicate, into the labelled cuvettes (5.2). Place the cuvettes in the incubator block (5.6) and prewarm to 38 °C for 5 min. Add, by means of the positive-displacement pipette (5.4), 0,075 ml of the prepared test portion (8.1) to all six cuvettes. Cover the cuvettes with the parafilm (5.7).

Vortex (5.8) for 5 s, or gently invert all six cuvettes to mix the contents and then return the cuvettes to the incubator block. Starting with calibrator solution A (4.4.1), perform the following calibration routine. Set the fluorimeter (5.1) to zero fluorescence using calibrator solution A (4.4.1) and then read and record the amount of fluorescence obtained with calibrator solution B and solution C against calibrator solution A. Wipe the outside of each cuvette with soft tissue before placing it in the fluorimeter. Once calibration is completed, proceed with the analysis of the samples.