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Milk and milk products — Determination of alkaline phosphatase activity —

Part 1: Fluorimetric method for milk and milk-based drinks

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

Partie 1: Méthode fluorimétrique pour le lait et les boissons à base de lait

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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).

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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 ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 302, *Milk and milk products — Methods of sampling and analysis*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement). It is being published jointly by ISO and IDF.

This fourth edition cancels and replaces the third edition (ISO 11816-1 | IDF 155-1:2013), which has been technically revised.

The main changes are as follows:

— the FLM200 (which has been discontinued) has been replaced by the FLM300 version;

— the instructions for use of the instrument and the flow of those instructions have been revised in accordance with FLM300, which has an upgraded user interface and electronics (there has been no change to the assay or the test procedure with the changes to the interface and software);

— the instrument now includes the heater block which was a separate item previously.

A list of all parts in the ISO 11816 | IDF 155 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.

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.

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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 P19 of the *Standing Committee on Analytical Methods for Processing Aids and Indicators* under the aegis of its project leader Mr Rick Zampa (US).

Milk and milk products — Determination of alkaline phosphatase activity —

Part 1:
Fluorimetric method for milk and milk-based drinks

1 Scope

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

This method is applicable to milk and milk-based drinks from cows, sheep and goats. It is also applicable to milk powder after reconstitution.

The instrument used for the determination of ALP can read activities up to 7 000 milliunits per litre (mU/l). If the activity is higher than 7 000 mU/l, it is diluted with ALP-free milk so as to obtain a measurement not higher than 7 000 mU/l.

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
activity of the enzyme present in the product, determined by the specified procedure

Note 1 to entry: The ALP activity is expressed as milliunits of enzyme activity per litre of sample (mU/l).

4 Principle

The ALP 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 ALP derived from the sample, undergoes hydrolysis of its phosphate radical, producing a highly fluorescent product. Fluorimetric measurement of ALP activity is measured at

38 °C over a 3 min period when using a Fluorophos®[1]. This includes pre-incubation of substrate and sample, followed by multiple kinetic readings of the reaction rate.

NOTE 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).

5 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

The reagents specified in 5.1 to 5.5 and the apparatus specified in 6.1 to 6.4 (except 6.3.3) comprise the Fluorophos® Test System[2]. The manufacturer can change the packaging configurations supplied with Fluorophos® Test system. The user should refer to the manufacturer's instructions for preparing reagents if different from those specified herein.

5.1 Fluorophos® substrate, in bottles, each containing 144 mg of Fluorophos® substrate powder, molar mass of 580 g/mol.

This is a non-fluorescent aromatic monophosphoric ester substrate, 2′-[2-benzothiazolyl]-6′-hydroxybenzothiazole phosphate (Fluorophos®).

The Fluorophos® substrate remains stable for two years from the date of manufacture, provided it is stored in unopened bottles at between 2 °C and 8 °C. Protect against light.

5.2 Substrate buffer solution, diethanolamine (DEA) buffer solution, c(DEA) = 2,4 mol/l, with pH-value 10,0, in bottles of 240 ml each.

The substrate buffer solution remains stable for two years from the date of manufacture, provided it is stored in unopened bottles at between 2 °C and 8 °C. Protect against light.

5.3 Working substrate.

Allow the Fluorophos® substrate (5.1) and the substrate buffer solution (5.2) to come to room temperature. Add the content of one bottle of substrate buffer solution (240 ml) (5.2) to that of one bottle of Fluorophos® substrate (144 mg) (5.1) and mix well by inversion for 3 min to create an approximately 1,0 millimolar (pH-value 10) solution. Use amber glass to protect against light.

Allow the obtained solution to stand at room temperature for at least 30 min prior to use.

Use the analogue-to-digital (A/D) test given in 9.2 to test the suitability of the ready-to-use working substrate. Do not use the working substrate if a reading above 1 200 FLU (fluorescence units) is obtained.

The working substrate remains stable for 60 days when protected from light and stored at between 2 °C and 8 °C, or for 8 h when stored at 38 °C.

NOTE The volume of the working substrate (240 ml) obtained is sufficient for approximately 115 tests.

1) Fluorophos® is the registered trademark of a product supplied by Advanced Instruments, LLC. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of the product named. Equivalent products may be used if they can be shown to lead to the same results.

2) The Fluorophos® Test System is the trade name of a product supplied by Advanced Instruments, LLC. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of the product named. Equivalent products may be used if they can be shown to lead to the same results.

5.4 Calibrator solutions, Fluoroyellow®[3] (FY) [2′-(2-benzothiazolyl)-6′-hydroxybenzothiazole] in substrate buffer solution (5.2).

The calibrator solutions remain stable for 18 months from the date of manufacture, provided they are stored in unopened bottles at between 2 °C and 8 °C. Mix gently prior to use to ensure optimal results.

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

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

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

5.5 Daily instrument control solution, containing $34{,}48 \times 10^{-3}$ µmol/l of Fluoroyellow®.

The daily instrument control solution remains stable for 18 months from the date of manufacture, provided it is stored in unopened bottles at between 2 °C and 8 °C. Mix gently prior to use to ensure optimal results.

6 Apparatus

Usual laboratory equipment and, in particular, the following shall be used.

6.1 Filter fluorimeter, with thermostatically controlled cuvette holder, capable of operating at 38 °C ± 1 °C and right-angle optics, allowing excitation at a wavelength of 440 nm and emission between 520 nm and 560 nm [e.g. Fluorophos® instrument].

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

6.3 Pipettes.

6.3.1 Fixed-volume dispenser, capable of dispensing 2,0 ml.

6.3.2 Positive-displacement or air-displacement pipette, of capacity 0,075 ml.

Follow strict instructions for the pipetting technique as this is a critical step in generating accurate results. Ensure that the piston of the pipette bore is tightly secured prior to use.

6.3.3 Pipettes, of capacity 2 ml and 3 ml.

6.4 Incubator block, capable of maintaining a temperature of 38 °C ± 1 °C, suitable for holding cuvettes.

6.5 Plastic paraffin film, (e.g. Parafilm®[4]) or other suitable laboratory-grade film**.**

6.6 Vortex mixer.

6.7 Water bath, capable of maintaining a temperature of 63 °C ± 1 °C and 95 °C ± 1 °C.

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

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3) Fluoroyellow® is the registered trademark of a product supplied by Advanced Instruments, LLC. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of the products named. Equivalent products may be used if they can be shown to lead to the same results.

4) Parafilm® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of this product.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this document. A recommended sampling method is given in ISO 707 | IDF 50.

8 Preparations

8.1 Alkaline phosphatase-free milk

Prepare ALP-free milk of the type to be tested by carefully dispensing the desired portion of milk into a test tube or suitable container, ensuring that no milk touches the rim or sides of the container.

Place the tube or container with the milk portion in the water bath (6.7) set at 95 °C. Preheat the milk portion to 95 °C, before starting its 5 min heating period at that temperature. Check the temperature by using a thermometer or thermistor probe placed in the centre of the tube or container. When the milk portion reaches 95 °C, immediately start its 5 min heating period. Cool the whole portion rapidly after the heating period.

Test the thus-treated milk portion to ensure that its ALP activity is less than 10 mU/l.

8.2 Preparation of the test sample

8.2.1 General

Carefully mix all test samples prior to use.

NOTE It is usually not necessary to prewarm test samples.

8.2.2 Pasteurized test samples

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

8.2.3 Dilution of test samples with high ALP values

Prepare dilutions of the test samples of milk using ALP-free milk (8.1) in order to bring their ALP levels within the analytical range of assay (< 7 000 mU/l). Mix the diluted solutions well.

9 Procedure

9.1 Verification of instrument performance

9.1.1 General

It is important to check instrument performance for drift, stray light and stability prior to analysing test samples. Follow good laboratory practice principles when operating the filter fluorimeter (6.1).

Quality control tests include the following:

a) the daily A/D test, used to check the proper functioning of the equipment;

b) the daily instrument control test, using the daily instrument control solution (5.5) to monitor any electronic or optical drift in the fluorimeter;

c) the use of external positive, negative and normal controls, described in 9.1.3, which are recommended for monitoring daily instrument precision parameters.