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

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

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*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 ISO 11816-1:2006*

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

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.

ISO 11816-1|IDF 155-1 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.

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This edition of ISO 11816-1|IDF 155-1 cancels and replaces ISO 11816-1:1997, 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*

## Foreword

**IDF (the International Dairy Federation)** is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the IDF National Committees casting a vote.

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.

ISO 11816-1|IDF 155-1 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by the Joint ISO-IDF Action Team *Characterization to heat treatment*, of the Standing Committee on *Minor components and characterization of physical properties*, under the aegis of its project leader, Mr F. Harding (UK).

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

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

### 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 pasteurized whole milk, semi-skimmed milk, skimmed milk and flavoured milks. The method is applicable for milk from cows, sheep and goats, and milk-based drinks.

The method is also suitable for the determination of high alkaline phosphatase activity in raw milk and heat-treated milk with activities of more than 2 000 mU/l after dilution of the sample as specified.

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### 2 Terms and definitions (standards.iteh.ai)

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

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#### 2.1

##### **alkaline phosphatase activity**

##### **APL activity**

activity of the alkaline phosphatase present in the product, determined by the procedure specified in this part of ISO 11816|IDF 155

NOTE The alkaline phosphatase activity is expressed as milliunits of enzyme activity per litre (mU/l).

#### 2.2

##### **unit of alkaline phosphatase activity**

amount of alkaline phosphatase enzyme that catalyses the transformation of 1  $\mu\text{mol}$  of substrate per minute

### 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, 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 measurement of alkaline phosphatase (ALP) activity is measured at 38 °C over a 3-min period when using the substrate. 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).

## 4 Reagents

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

**4.1 Fluorophos<sup>®</sup> substrate** <sup>1)</sup>, in bottles, each containing 144 mg of Fluorophos<sup>®</sup> substrate powder.

This is a non-fluorescent aromatic monophosphoric ester substrate, 2'-[2-benzothiazolyl]-6'-hydroxybenzothiazole phosphate (Fluorophos<sup>®</sup>). The Fluorophos<sup>®</sup> substrate remains stable for 2 years when stored in unopened bottles at between 2 °C and 8 °C.

**4.2 Substrate buffer solution**, diethanolamine (DEA) buffer solution,  $c(\text{DEA}) = 2,4 \text{ mol/l}$ , with pH 10,0, in bottles of 240 ml each.

The substrate buffer solution remains stable for 2 years when stored in unopened bottles at between 2 °C and 8 °C.

### 4.3 Working substrate

Allow the Fluorophos<sup>®</sup> substrate (4.1) and the substrate buffer solution (4.2) to come to room temperature. Add the content of one bottle of substrate buffer solution (240 ml) (4.2) to that of one bottle Fluorophos<sup>®</sup> substrate (144 mg) (4.1) and mix well by inversion for 3 min. Use amber glass to protect against light.

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

Use the analog-to-digital test given in 8.4.1.1 to test the suitability of the ready to use working substrate.

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. Do not use the working substrate if a reading above 1 200 is obtained (see 8.4.1.1.5).

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

**4.4 Working calibrator solutions**, Fluoroyellow<sup>®</sup>(FY) [2'-(2-benzothiazolyl)-6'-hydroxybenzothiazole] in DEA buffer (4.2).

The working calibrator solutions remain stable for 18 months when stored at between 2 °C and 8 °C.

**4.4.1 Calibrator solution A**, containing 0 µmol/l of Fluoroyellow<sup>®</sup>.

**4.4.2 Calibrator solution B**, containing  $17,24 \times 10^{-3} \text{ µmol/l}$  of Fluoroyellow<sup>®</sup>.

**4.4.3 Calibrator solution C**, containing  $34,48 \times 10^{-3} \text{ µmol/l}$  of Fluoroyellow<sup>®</sup>.

**4.5 Daily instrument control solution**, containing  $34,48 \times 10^{-3} \text{ µmol/l}$  of Fluoroyellow<sup>®</sup>.

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1) The reagents specified in 4.1 to 4.5 and the apparatus specified in 5.1 to 5.4 (except 5.3.3) are available as Fluorophos Test System from Advanced Instruments, Inc., Two Technology Way, Norwood, Massachusetts 02062, USA. The manufacturer may change packaging configurations supplied with the Fluorophos Test System. The user should refer to the manufacturer's instructions for preparing reagents if different from those specified herein. 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 document and does not constitute an endorsement by either ISO or IDF of these products.



## 5 Apparatus

Usual laboratory equipment and, in particular, the following.

**5.1 Filter fluorimeter**, with thermostatically controlled cuvette holder, capable of operating at  $38\text{ °C} \pm 1\text{ °C}$  and right-angle optics, allowing excitation at a wavelength of 440 nm and emission at between 520 nm and 560 nm [e.g. Fluorophos<sup>®</sup> instrument <sup>1)</sup>]. Measurements should be optimized according to the manufacturers instructions.

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

**5.3 Pipettes.**

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

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

**5.3.3 Pipette**, of capacity 2 ml.

**5.4 Incubator block**, capable of maintaining a temperature of  $38\text{ °C} \pm 1\text{ °C}$ , suitable for holding cuvettes.

**5.5 Parafilm** <sup>2)</sup>, or other suitable laboratory-grade film.

**5.6 Vortex mixer.**

**5.7 Water bath**, capable of maintaining a temperature of  $63\text{ °C} \pm 1\text{ °C}$  and  $95\text{ °C} \pm 1\text{ °C}$ .

**5.8 One-mark volumetric flasks**, of capacity 100 ml.

## 6 Sampling

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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 part of ISO 11816|IDF 155. A recommended sampling method is given in ISO 707|IDF 50.

## 7 Preparations

### 7.1 Alkaline phosphatase-free milk

Prepare phosphatase-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 (5.7) set at  $95\text{ °C}$ . Preheat the milk portion to  $95\text{ °C}$ , 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  $95\text{ °C}$ , immediately start the 5-min heating period. Cool the whole rapidly after the heating period.

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

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2) Parafilm is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 11816|IDF 155 and does not constitute an endorsement by either ISO or IDF of this product.