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**Milk and milk-based drinks —  
Determination of alkaline phosphatase  
activity — Enzymatic photo-activated  
system (EPAS) method**

*Lait et boissons à base de lait — Détermination de l'activité de la  
phosphatase alcaline — Méthode par un système de photoactivation  
enzymatique*



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## Contents

Page

Foreword.....	iv
1 Scope .....	1
2 Terms and definitions.....	1
3 Principle.....	1
4 Reagents.....	2
5 Apparatus .....	3
6 Sampling.....	3
7 Preparations .....	4
7.1 Test samples .....	4
7.2 Alkaline phosphatase-free milk.....	4
8 Procedure (see Annex A).....	4
8.1 Calibration .....	4
8.2 Calibration adjustment.....	6
8.3 Control tests and calibration check.....	9
8.4 Determination.....	10
8.5 Heat-resistant microbial alkaline phosphatase control.....	10
9 Calculation and expression of results.....	10
9.1 General.....	10
9.2 Manual calculation.....	10
9.3 Expression of results .....	11
10 Precision.....	11
10.1 Interlaboratory test .....	11
10.2 Repeatability.....	11
10.3 Reproducibility.....	12
11 Test report .....	12
Annex A (informative) Procedure flowchart .....	13
Annex B (informative) Results of interlaboratory trials.....	14
Bibliography .....	16

## 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 22160|IDF 209 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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## 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 22160|IDF 209 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 on *Heat treatment*, of the Standing Committee on *Minor components & characterization of physical properties*, under the aegis of its project leader, Mr R. Salter (US).

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# Milk and milk-based drinks — Determination of alkaline phosphatase activity — Enzymatic photo-activated system (EPAS) method

## 1 Scope

This International Standard specifies a method for the determination of the alkaline phosphatase activity in pasteurized whole milk, semi-skimmed milk, skimmed milk, cream and flavoured milks using a chemiluminescent (EPAS) method.

The method is applicable to milk and milk-based drinks from cows, sheep, buffalo and goats.

The method is also suitable for any liquid-based sample if diluted in such a way that the diluted alkaline phosphatase activity has less than 7 000 milliunits per litre.

NOTE There has been a successful collaborative trial with cow, sheep, buffalo, and goat whole milk, as well as skimmed cow milk (< 0,5 % fat), 20 % fat cream, and 2 % fat chocolate milk (all mass fractions).

## 2 Terms and definitions

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

### 2.1

#### alkaline phosphatase activity

##### ALP

activity of the alkaline phosphatase present in the product, determined according to the procedure described in this International Standard

NOTE The alkaline phosphatase activity is expressed as milliunits of enzyme activity per litre (mU/l) [4], [5].

### 2.2

#### unit of alkaline phosphatase activity

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

## 3 Principle

The alkaline phosphatase activity is measured by photo-activation of the hydrolysed product followed by an instrumental measurement of photo-activation. In the presence of alkaline phosphatase, a stable aromatic dioxetane-phosphate substrate is hydrolysed at  $35\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  to produce a photo-activated (chemiluminescent) product. The photo-activation of the product is amplified by a macromolecular enhancing component. The hydrolysis reaction is stopped after a specified incubation time (3 min). The amount of chemiluminescent product thus produced is measured and converted to enzyme units by a luminometer. Luminometer calibration is based on calibration using tablets with known enzyme activity.