



**SLOVENSKI STANDARD**  
**SIST ISO 3356:2011**

**01-junij-2011**

**Nadomešča:**  
**SIST ISO 3356:2001**

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**Mleko - Določevanje alkalne fosfataze**

Milk -- Determination of alkaline phosphatase

**iTeh STANDARD PREVIEW**  
Lait -- Détermination de la phosphatase alcaline  
**(standards.iteh.ai)**

**Ta slovenski standard je istoveten z: ~~SIST ISO 3356:2009~~ ISO 3356:2009**

<https://standards.iteh.ai/catalog/standards/sist/9c957c3b-16ab-4550-963c-e3890620b706/sist-iso-3356-2011>

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**ICS:**

67.100.10	Mleko in predelani mlečni proizvodi	Milk and processed milk products
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**SIST ISO 3356:2011**

**en,fr**

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ISO  
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63

Second edition  
2009-03-01

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**Milk — Determination of alkaline  
phosphatase**

*Lait — Détermination de la phosphatase alcaline*

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**ISO 3356:2009(E)**  
**IDF 63:2009(E)**

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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 3356|IDF 63 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 second edition of ISO 3356|IDF 63 cancels and replaces the first edition of ISO 3356:1975, which has been technically revised.

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ISO 3356:2009(E)  
IDF 63:2009(E)

## Foreword

**IDF (the International Dairy Federation)** is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented at 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 IDF National Committees casting a vote.

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ISO 3356|IDF 63 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by ISO and IDF.

All work was carried out by the Joint ISO-IDF Action Team on *Heat treatment* of the Standing Committee on *Minor components and characterization of physical properties* under the aegis of its project leader, Mrs. M. Nicolas (FR).

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# Milk — Determination of alkaline phosphatase

**WARNING** — The use of this International Standard may involve hazardous materials, operations and reagents. Persons using this International Standard should be familiar with normal laboratory practice. This International Standard does not purport to address all the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

## 1 Scope

This International Standard specifies a method for the determination of alkaline phosphatase activity in milk.

The method applies to alkaline phosphatase activities not less than 1 µg of phenol per millilitre.

The method is also suitable for the determination of alkaline phosphatase activity in milk powder, buttermilk and buttermilk powder, whey and whey powder.

## iTeh STANDARD PREVIEW

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

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

[SIST ISO 3356:2011](https://standards.iteh.ai/catalog/standards/sist/9c957c3b-16ab-4550-963c-c3890620b706/sist-iso-3356-2011)

**2.1** <https://standards.iteh.ai/catalog/standards/sist/9c957c3b-16ab-4550-963c-c3890620b706/sist-iso-3356-2011>  
**alkaline phosphatase activity**  
**ALP activity**

(alkaline phosphatase activity in milk) quantity of phenol liberated by the sample determined according to the procedure specified in this International Standard

**NOTE** The alkaline phosphatase activity is expressed as the quantity of phenol, in micrograms, liberated by 1 ml of the sample or of reconstituted sample, under the conditions specified in this International Standard. Other International Standards (e.g. ISO 11816-1|IDF 155-1<sup>[6]</sup>, ISO 22160|IDF 209<sup>[7]</sup>) express alkaline phosphatase activity in milliunits per litre. The literature gives information on the equivalence of the different units used to express the alkaline phosphatase activity.

## 3 Principle

The sample is diluted with a buffer at pH 10,6 and incubated at a temperature of 37 °C for 1 h. Under these conditions, any alkaline phosphatase present in the sample liberates phenol from the disodium phenylphosphate added. The phenol liberated reacts with a quinoneimide (dibromoquinonechlorimide) to produce dibromoindophenol (blue colour) which is measured photometrically at 610 nm.

## 4 Reagents

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

**ISO 3356:2009(E)**  
**IDF 63:2009(E)**

**4.1 Barium borate-hydroxide buffer solution**

Dissolve 25,0 g of barium hydroxide [Ba(OH)<sub>2</sub>·8H<sub>2</sub>O], carbonate free, in water in a 500 ml one-mark volumetric flask (5.8). Make up to the mark with water and mix.

Dissolve 11,0 g of boric acid (H<sub>3</sub>BO<sub>3</sub>) in water in another 500 ml one-mark volumetric flask (5.8). Make up to the mark with water and mix.

Warm both solutions to 50 °C. Add one to the other and mix by stirring. Cool the solution obtained rapidly to about 20 °C. Adjust the pH of the solution, if necessary, to 10,6 ± 0,1 with an additional amount of barium hydroxide solution. Filter the solution through filter paper (5.10).

Store the filtered barium borate-hydroxide buffer solution in a tightly stoppered container. Before use, dilute the buffer solution with an equal volume of water.

**4.2 Colour development buffer solutions**

**4.2.1 Colour buffer solution I**

Dissolve 6,0 g of sodium metaborate (NaBO<sub>2</sub>) or 12,6 g of NaBO<sub>2</sub>·4H<sub>2</sub>O, and 20,0 g of sodium chloride (NaCl) in water in a 1 000 ml one-mark volumetric flask (5.8). Make up to the mark with water and mix.

**4.2.2 Colour buffer solution II**

Transfer 10 ml of buffer solution I (4.2.1) to a 100 ml one-mark volumetric flask (5.8). Make up to the mark with water and mix.

**4.3 Buffer substrate solution**

**4.3.1** Disodium phenylphosphate dihydrate (Na<sub>2</sub>C<sub>6</sub>H<sub>5</sub>PO<sub>4</sub>·2H<sub>2</sub>O), containing no more than 0,01 % mass fraction phenol.

**4.3.2** Dissolve 0,1 g of disodium phenylphosphate dihydrate (4.3.1) in 100 ml of diluted barium borate-hydroxide buffer solution (4.1).

**4.4 Zinc-copper precipitant solution**

Dissolve 3,0 g of zinc sulfate (ZnSO<sub>4</sub>·7H<sub>2</sub>O) and 0,6 g of copper sulfate (CuSO<sub>4</sub>·5H<sub>2</sub>O) in water in a 100 ml one-mark volumetric flask (5.8). Make up to the mark with water and mix.

**4.5 2,6-Dibromoquinonechloroimide (BQC) solution, Gibb's reagent.**

Dissolve 40 mg ± 1 mg of BQC (C<sub>6</sub>H<sub>2</sub>Br<sub>2</sub>ClNO) in 10 ml of ethanol 96 % volume fraction.

Store the solution in a dark coloured bottle at 4 °C ± 2 °C. Reject if discoloured or more than 1 month old.

**4.6 Copper sulfate solution**

Dissolve 0,05 g of copper sulfate (CuSO<sub>4</sub>·5H<sub>2</sub>O) in water in a 100 ml one-mark volumetric flask (5.8). Make up to the 100 ml mark with water and mix.



**4.7 Sodium hydroxide solution**,  $c(\text{NaOH}) = 0,5 \text{ mol/l}$ .

#### 4.8 Phenol standard solutions

##### 4.8.1 Phenol standard stock solution

Transfer a weighed amount of  $200 \text{ mg} \pm 2 \text{ mg}$  of anhydrous phenol of purity higher than 99,5 % mass fraction into a 100 ml one-mark volumetric flask (5.8). Dissolve the phenol in water. Make up to the mark with water and mix.

The phenol standard stock solution remains stable at  $4 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$  for 6 weeks.

##### 4.8.2 Phenol standard working solutions

Pipette 10 ml of phenol standard stock solution (4.8.1) into a 100 ml one-mark volumetric flask (5.8). Make up to the mark with water and mix (1 ml contains  $200 \text{ } \mu\text{g}$  of phenol).

Use the diluted standard solution to prepare the appropriate phenol standard working solutions, containing  $2 \text{ } \mu\text{g}$ ,  $5 \text{ } \mu\text{g}$ ,  $10 \text{ } \mu\text{g}$  and  $20 \text{ } \mu\text{g}$  of phenol per millilitre respectively.

## 5 Apparatus

Usual laboratory equipment and, in particular, the following.

**5.1 Analytical balance**, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.

**5.2 Photometer**, suitable for measuring at a wavelength of 610 nm.

**5.3 Water bath**, capable of being maintained at  $37 \text{ }^\circ\text{C} \pm 0,1 \text{ }^\circ\text{C}$  under thermostatic control.

**5.4 Boiling water bath**.

**5.5 Vortex mixer**.

**5.6 Pipettes**, capacities 0,1 ml, 1 ml, 5 ml and 10 ml, ISO 648<sup>[1]</sup>, class A.

**5.7 Glass test tubes**, of appropriate volumes, with closures made from phenolic-free liners.

**5.8 One-mark volumetric flasks**, capacities 100 ml, 500 ml and 1000 ml, ISO 1042<sup>[3]</sup>, class A.

**5.9 Glass funnels**, diameters about 60 mm and about 100 mm.

**5.10 Filter paper**, fast grade, diameters about 110 mm and about 185 mm.

## 6 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 International Standard. A recommended sampling method is given in ISO 707 | IDF 50<sup>[2]</sup>.

Store the test sample in such a way that deterioration and change in composition are prevented.