

**Milk and milk products - Determination of alkaline phosphatase activity - Part 1: Fluorimetric method for milk and milk-based drinks (ISO 11816-1:2013)**

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English Version

## Milk and milk products - Determination of alkaline phosphatase activity - Part 1: Fluorimetric method for milk and milk-based drinks (ISO 11816-1:2013)

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 11816-1:2013)

Milch und Milcherzeugnisse - Bestimmung der Aktivität der alkalischen Phosphatase - Teil 1: Fluorimetrisches Verfahren für Milch und flüssige Milchprodukte (ISO 11816-1:2013)

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**CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels**

## Foreword

This document (EN ISO 11816-1:2013) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 302 "Milk and milk products - Methods of sampling and analysis" the secretariat of which is held by NEN.

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of an identical text or by endorsement, at the latest by May 2014, and conflicting national standards shall be withdrawn at the latest by May 2014.

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### Endorsement notice

The text of ISO 11816-1:2013 has been approved by CEN as EN ISO 11816-1:2013 without any modification.

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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 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 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 alkaline phosphatase-free milk (7.1) so as to obtain a measurement not higher than 7 000 mU/l.

### 2 Terms and definitions

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

#### 2.1

##### **alkaline phosphatase (ALP) activity**

activity of the alkaline phosphatase present in the product, determined by the specified procedure

Note 1 to entry: The alkaline phosphatase activity is expressed as milliunits of enzyme activity per litre of sample (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 Fluorophos®. 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.