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Milk and milk products — Determination of alkaline phosphatase activity — Fluorimetric microplate method

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TECHNICAL SPECIFICATION

ISO/TS 4985:2023(E)
IDF/RM 255:2023(E)

Milk and milk products — Determination of alkaline phosphatase activity — Fluorimetric microplate method

1 Scope

This document specifies a fluorimetric microplate method for the determination of alkaline phosphatase (ALP, EC 3.1.3.1)[5] activity in raw and heat-treated whole milk, semi-skimmed milk, skimmed milk, cream, flavoured milks and cheeses.

This method is applicable to milk and milk-based drinks from cows, sheep and goats. Although the method was not tested in milk from other species, it can also be applicable to milk from other species with a similar composition to cow, sheep or goat milk, such as milk from buffalo and camelids. It is also applicable to milk powder after reconstitution and soft, semi-hard and hard cheeses provided that the mould is only on the surface of the cheese and not also in the inner part (e.g. blue veined cheeses). For large hard cheeses, specific conditions of sampling apply (see Clause 7).

NOTE This method was adapted from Reference [6].

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
amount of enzyme that is capable of catalysing the transformation of 1 µmol of substrate per minute under the conditions of the specified procedure

Note 1 to entry: The ALP activity in milk is expressed as milliunits of enzyme activity per litre of sample (mU/l) and as milliunits of enzyme activity per gram of sample (mU/g) in the case of cheese.

4 Principle

The ALP activity of the sample is measured by a continuous fluorimetric direct kinetic assay. A non-fluorescent aromatic monophosphoric substrate, 4-methylumbelliferone phosphate (4-MUP), in the presence of any ALP derived from the sample, undergoes hydrolysis of its phosphate, producing the highly fluorescent product 4-methylumbelliferone (4-MU). Fluorimetric measurement of ALP activity is measured at 37 °C, over a 15 min period.

The measured fluorescence is proportional to the concentration of the emitted fluorescent product and is used to calculate the enzyme activity.

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