
**Microbiology of the food chain —
Preparation of test samples, initial
suspension and decimal dilutions for
microbiological examination —**

Part 5:

**Specific rules for the preparation of
milk and milk products**

iTeh STANDARD PREVIEW
(standards.iteh.ai)

*Microbiologie de la chaîne alimentaire — Préparation des
échantillons, de la suspension mère et des dilutions décimales en vue
de l'examen microbiologique*

<https://standards.iteh.ai/catalog/standards/sist/37-4c0f-8311-572b5c1ba451-6887-5-2020>

*Partie 5: Règles spécifiques pour la préparation du lait et des produits
laitiers*



iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 6887-5:2020

<https://standards.iteh.ai/catalog/standards/sist/5309f53-c3f7-4c0f-8311-ef72b5c1ba4c/iso-6887-5-2020>



COPYRIGHT PROTECTED DOCUMENT

© ISO 2020

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

	Page
Foreword	iv
1 Scope	1
2 Normative references	1
3 Terms and definitions	2
4 Principle	2
5 Diluents	2
5.1 List of diluents.....	2
5.2 Distribution and sterilization of the diluents.....	5
5.3 Performance testing for diluents.....	5
6 Apparatus	6
7 Sampling	6
8 General procedures	6
8.1 General.....	6
8.2 Frozen products.....	6
8.3 Hard and dry products.....	7
8.4 Liquid and non-viscous products.....	7
8.5 Multi-component products.....	7
8.6 Acidic products.....	7
8.7 High-fat foods (fat content > 20 % mass fraction).....	7
9 Specific procedures	7
9.1 Milk and liquid milk products.....	7
9.2 Dehydrated milk, dehydrated sweet whey, dehydrated acid whey, dehydrated buttermilk and lactose.....	7
9.3 Cheese and cheese products.....	8
9.4 Acid casein, lactic casein, rennet casein and caseinate.....	8
9.4.1 General case.....	8
9.4.2 Special case: Rennet casein.....	8
9.5 Butter.....	8
9.6 Milk-based ice-cream.....	9
9.7 Milk-based custard, desserts and sweet cream (pH > 5).....	9
9.8 Milk-based fermented milks, yogurt, probiotics milk products and sour cream (pH < 5).....	9
9.9 Dehydrated milk-based infant foods with or without probiotics.....	9
10 Further decimal dilutions	10
Bibliography	11

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 6887-5:2010), which has been technically revised. The main changes compared with the previous edition are as follows:

- the document has been aligned with ISO 6887-1, ISO 6887-2, ISO 6887-3 and ISO 6887-4;
- cross references have been added to ISO 6887-1 where relevant.

A list of all parts in the ISO 6887 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination —

Part 5: Specific rules for the preparation of milk and milk products

WARNING — The use of this document can involve hazardous materials, operations and equipment. It is the responsibility of the user of this document to establish appropriate safety and health practices and to determine the applicability of regulatory limitations before use.

1 Scope

This document specifies rules for the preparation of samples of milk and milk products and their suspensions for microbiological examination when the samples require a different preparation from the general methods specified in ISO 6887-1.

This document excludes the preparation of samples for both enumeration and detection test methods where preparation details are specified in the relevant International Standards.

This document is intended to be used in conjunction with ISO 6887-1.

This document is applicable to: <https://standards.iso.org/standards/catalog/standards/sist/5309f53-c3f7-4c0f-8311-ef72b5c1ba4c/iso-6887-5-2020>

- a) milk and liquid milk products;
- b) dehydrated milk products;
- c) cheese and cheese products;
- d) casein and caseinates;
- e) butter;
- f) milk-based ice-cream;
- g) milk-based custard, desserts and sweet cream;
- h) fermented milks, yogurt, probiotics milk products and sour cream;
- i) dehydrated milk-based infant foods, with or without probiotics.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887-1, *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 6887-1 apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

4 Principle

The general principles for sample preparation and subsequent steps are detailed in ISO 6887-1. This document describes specific sample preparation for milk and milk products.

5 Diluents

5.1 List of diluents

Follow current laboratory practices as specified in ISO 7218. The composition of culture media and reagents and their preparation are specified in ISO 6887-1 or in the following procedures.

ISO 6887-5:2020

5.1.1 Basic materials. See ISO 6887-1. <http://std.iso.org/iso/catalog/standards/sist/5309f53-c3f7-4c0f-8311-ef72b5c1ba4c/iso-6887-5-2020>

5.1.2 Diluents for general use. Peptone salt solution, buffered peptone water and double-strength buffered peptone water are described in ISO 6887-1.

5.1.2.1 Quarter-strength Ringer's solution.

5.1.2.1.1 Composition

Sodium chloride (NaCl) (CAS No. 7647-14-5)	2,25 g
Potassium chloride (KCl) (CAS No. 7447-40-7)	0,105 g
Calcium chloride anhydrous (CaCl ₂) (CAS No. 10043-52-4)	0,06 g ^a
Sodium hydrogen carbonate (NaHCO ₃) (Cas No. 144-55-8)	0,05 g
Water	1 000 ml

^a Alternatively, use 0,12 g of CaCl₂·6H₂O (CAS No. 10035-04-8).

5.1.2.1.2 Preparation

Dissolve the salts in the water. Adjust the pH, if necessary, so that after sterilization it is 6,9 ± 0,2 at 25 °C.

5.1.2.2 Peptone solution.**5.1.2.2.1 Composition**

Enzymatic digest of casein	1,0 g
Water	1 000 ml

5.1.2.2.2 Preparation

Dissolve the peptone in the water. Adjust the pH, if necessary, so that after sterilization it is $7,0 \pm 0,2$ at 25 °C.

5.1.2.3 Phosphate buffer solution.**5.1.2.3.1 Composition**

Potassium dihydrogen phosphate (anhydrous) (KH_2PO_4) (CAS No. 7778-77-0)	42,5 g
Water	1 000 ml

5.1.2.3.2 Preparation

Dissolve the salt in 500 ml of water. Adjust the pH, if necessary, so that after sterilization it is $7,2 \pm 0,2$ at 25 °C. Dilute to 1 000 ml with the remaining water.

Store the stock solution under refrigerated conditions.

Add 1 ml of this stock solution to 1 000 ml of water for use as diluent.

5.1.3 Diluents for special purposes. These diluents shall only be used for the preparation of initial suspensions.

5.1.3.1 Sodium citrate solution.**5.1.3.1.1 Composition**

Trisodium citrate dihydrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) (CAS No. 6132-04-3)	20,0 g
Water	1 000 ml

5.1.3.1.2 Preparation

Dissolve the salt in water by heating, if necessary, on a hotplate (6.3) at a temperature between 45 °C and 50 °C. Adjust the pH, if necessary, so that after sterilization it is $7,5 \pm 0,2$ at 25 °C.

5.1.3.1.3 Application

This solution is used for cheese and (roller-)dried milk, and some caseinates.

5.1.3.2 Dipotassium hydrogen phosphate solution.

5.1.3.2.1 Composition

Dipotassium hydrogen phosphate (K_2HPO_4) (CAS No. 7758-11-4)	20,0 g
Water	1 000 ml

5.1.3.2.2 Preparation

Dissolve the salt in the water by heating, if necessary, on a hotplate (6.3) at a temperature between 45 °C and 50 °C. For acid whey powder, adjust the pH so that for the primary dilution after sterilization it is $8,4 \pm 0,2$ at 25 °C. For cheese, roller-dried milk, fermented milk, yogurt, caseinates and sour cream, adjust the pH so that after sterilization it is $7,5 \pm 0,2$ at 25 °C.

5.1.3.2.3 Application

This solution is used for cheese, (roller-)dried milk, fermented milk, yogurt, some caseinates, dehydrated acid whey, and sour cream.

5.1.3.3 Dipotassium hydrogen phosphate solution with antifoam agent.

5.1.3.3.1 Composition

5.1.3.3.1.1 Dipotassium hydrogen phosphate solution

Prepare according to 5.1.3.2.

5.1.3.3.1.2 Antifoam stock solution

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 6887-5:2020

https://standards.iteh.ai/catalog/standards/sist/5309f53-c3f7-4c0f-8311-ef72b5c1ba4c/iso-6887-5-2020

5.1.3.3.1.2.1 Composition

Polyethylene glycol 2000 (CAS No. 25322-68-3)	1 g
Water	100 ml

5.1.3.3.1.2.2 Preparation

Dissolve the polyethylene glycol 2000 in the water by mixing.

5.1.3.3.2 Preparation

Add 1 ml of the antifoam stock solution (5.1.3.3.1.2) to 1 l of the K_2HPO_4 solution (5.1.3.3.1.1). Adjust the pH so that for the primary dilution of both acid and lactic casein, after sterilization, it is $8,4 \pm 0,2$ at 25 °C, and for rennet casein, after sterilization, it is $7,5 \pm 0,2$ at 25 °C.

5.1.3.3.3 Application

This solution is used for acid casein, lactic casein and rennet caseins.

5.1.3.4 Tripolyphosphate solution.

5.1.3.4.1 Composition

Sodium tripolyphosphate pentabasic ($\text{Na}_5\text{O}_{10}\text{P}_3$) (CAS No. 7758-29-4)	20,0 g
Water	1 000 ml

5.1.3.4.2 Preparation

Dissolve the salt in the water by heating slightly on a hotplate (6.3), if necessary. The solution may be stored at a temperature of $5\text{ }^\circ\text{C} \pm 3\text{ }^\circ\text{C}$ for a maximum of one month.

5.1.3.4.3 Application

This solution is used as alternative diluent for rennet caseins that are difficult to dissolve.

5.1.3.5 Diluent for general use with α -amylase solution.

5.1.3.5.1 Preparation

For a 25 g test portion, add 12,5 mg of α -amylase (EC 3.2.1.1, see Reference [1]) with a specific activity of approximately 400 units (= 6,7 μkat) per milligram to 225 ml of the diluent for general use (5.1.2). Use amounts in the same proportion for preparation of other test portions (e.g. for a 10 g test portion, add 5 mg of α -amylase to 90 ml of the diluent for general use).

NOTE The unit (often called the "international unit" or "standard unit") is defined as the amount of enzyme that catalyses the transformation of 1 μmol of substrate per minute under standard conditions.

[ISO 6887-5:2020](https://standards.iteh.ai/catalog/standards/sist/5309f53-c3f7-4c0f-8311-ef72b5c1ba4c/iso-6887-5-2020)

5.1.3.5.2 Application

This solution may be used for foods containing starch, when the primary dilution presents a solubility problem.

NOTE An example of a solubility issue is when the initial suspension is too thick to mix or pipette.

5.2 Distribution and sterilization of the diluents

Follow ISO 6887-1.

5.3 Performance testing for diluents

For performance testing of all diluents, follow the procedures as specified in ISO 11133 and as described in [Table 1](#).