# SVENSK STANDARD
# SS-EN ISO 16212:2017

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## Kosmetika – Mikrobiologi – Räkning av jäst och mögel (ISO 16212:2017)

## Cosmetics – Microbiology – Enumeration of yeast and mould (ISO 16212:2017)

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Europastandarden EN ISO 16212:2017 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN ISO 16212:2017.

Denna standard ersätter SS-EN ISO 16212:2011, utgåva 1.

The European Standard EN ISO 16212:2017 has the status of a Swedish Standard. This document contains the official version of EN ISO 16212:2017.

This standard supersedes the Swedish Standard SS-EN ISO 16212:2011, edition 1.



*Upplysningar om sakinnehållet i standarden lämnas av SIS, Swedish Standards Institute, telefon 08-555 520 00. Standarder kan beställas hos SIS Förlag AB som även lämnar allmänna upplysningar om svensk och utländsk standard.*

*Information about the content of the standard is available from the Swedish Standards Institute (SIS), telephone +46 8 555 520 00. Standards may be ordered from SIS Förlag AB, who can also provide general information about Swedish and foreign standards.*

Denna standard är framtagen av kommittén för Kosmetik samt analysmetoder för allergener, SIS/TK 495.

Har du synpunkter på innehållet i den här standarden, vill du delta i ett kommande revideringsarbete eller vara med och ta fram andra standarder inom området? Gå in på www.sis.se - där hittar du mer information.

# EUROPEAN STANDARD
# NORME EUROPÉENNE
# EUROPÄISCHE NORM

# EN ISO 16212

July 2017

ICS 07.100.40

Supersedes EN ISO 16212:2011

English Version

## Cosmetics - Microbiology - Enumeration of yeast and mould (ISO 16212:2017)

Cosmétiques - Microbiologie - Dénombrement des levures et des moisissures (ISO 16212:2017)

Kosmetische Mittel - Mikrobiologie - Zählung von Hefen und Schimmelpilzen (ISO 16212:2017)

This European Standard was approved by CEN on 26 April 2017.

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



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# European foreword

This document (EN ISO 16212:2017) has been prepared by Technical Committee ISO/TC 217 "Cosmetics" in collaboration with Technical Committee CEN/TC 392 “Cosmetics” the secretariat of which is held by AFNOR.

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 January 2018 and conflicting national standards shall be withdrawn at the latest by January 2018.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 16212:2011.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

**Endorsement notice**

The text of ISO 16212:2017 has been approved by CEN as EN ISO 16212:2017 without any modification.

# Cosmetics — Microbiology — Enumeration of yeast and mould

## 1 Scope

This document gives general guidelines for enumeration of yeast and mould present in cosmetics by counting the colonies on selective agar medium after aerobic incubation.

In order to ensure product quality and safety for consumers, it is advisable to perform an appropriate microbiological risk analysis to determine the types of cosmetic products to which this document is applicable. Products considered to present a low microbiological risk (see ISO 29621) include those with low water activity or extreme pH values, hydro-alcoholic products, etc.

Because of the large variety of cosmetic products within this field of application, this method might not be suited to some products in every detail (e.g. certain water-immiscible products). Other methods (e.g. automated) can be substituted for the tests presented here provided that their equivalence has been demonstrated or the method has been otherwise shown to be suitable.

Yeast enumerated can be identified using suitable identification tests, for example, tests described in the standards listed in the Bibliography. Mould enumerated can be identified by other appropriate methods, if necessary.

## 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 21148, *Cosmetics — Microbiology — General instructions for microbiological examination*

EN 12353, *Chemical disinfectants and antiseptics — Preservation of test organisms used for the determination of bactericidal (including Legionella), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity*

## 3 Terms and definitions

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

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

— ISO Online browsing platform: available at http://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

**3.1**
**yeast**
single-cell fungus, which multiplies mainly vegetatively by budding, able to grow under the test conditions specified in this document

**3.2**
**mould**
mycelium forming microfungus, including spores and conidia, able to grow under the test conditions specified in this document

**3.3**
**product**
portion of an identified cosmetic product received in the laboratory for testing

**3.4**
**sample**
portion of the *product* (3.3) (at least 1 g or 1 ml) that is used in the test to prepare the initial suspension

**3.5**
**initial suspension**
suspension (or solution) of the *sample* (3.4) in a defined volume of an appropriate enrichment broth

**3.6**
**sample dilution**
dilution of the *initial suspension* (3.5)

# 4 Principles

## 4.1 General

This method involves enumeration of colonies on a selective agar medium. The possible inhibition of fungal growth by the sample shall be neutralized to allow the detection of viable microorganisms[5]. In all cases and whatever the methodology, the neutralization of the antifungicidal properties of the product shall be checked and demonstrated[6][8][9].

## 4.2 Plate count

Plate count consists of the following steps.

— Preparation of poured plates, or spread plates, using a specified culture medium, and inoculation of the plates using a defined quantity of the initial suspension or dilution of the product.

— Aerobic incubation of the plates at 25 °C ± 2,5 °C for 3 d to 5 d.

— Counting of the number of colony-forming units (CFU) and calculation of the amount of yeast and mould per millilitre or per gram of product.

NOTE An alternative condition for incubation is 22,5 °C ± 2,5 °C for 5 d to 7 d using the culture medium without antibiotic.

## 4.3 Membrane filtration

Membrane filtration consists of the following steps.

— Transfer a suitable amount of the sample, prepared as described in Clause 12, in the filtration apparatus, wetted with a small volume of an appropriate sterile diluent. Filter immediately and wash according to the described procedure (see 12.3.4). Transfer the membrane filter onto the surface of the specified agar medium as specified in ISO 21148.

— Aerobic incubation of the membranes at 25 °C ± 2,5 °C for 3 d to 5 d.

— Counting of the number of colony-forming units (CFU) and calculation of the amount of yeast and mould per millilitre or per gram of product.

NOTE An alternative condition for incubation is 22,5 °C ± 2,5 °C for 5 d to 7 d using the culture medium without antibiotic.

# 5 Diluents, neutralizers and culture media

## 5.1 General

General instructions are given in ISO 21148. When water is mentioned in this document, use distilled water or purified water as specified in ISO 21148.

The following diluents, neutralizers and culture media are suitable for enumeration of yeasts and moulds. Other diluents, neutralizers and culture media may be used if they have been demonstrated to be suitable for use.

## 5.2 Neutralizing diluents and diluents

### 5.2.1 General

The diluent is used to disperse the sample. It may contain neutralizers if the sample to be tested has antifungicidal properties. The efficacy of the neutralization shall be demonstrated before the determination of the count (see Clause 12). Information relative to suitable neutralizers is given in Annex D.

### 5.2.2 Neutralizing diluent

#### 5.2.2.1 Fluid casein digest–soy lecithin–polysorbate 20 medium (SCDLP 20 broth)

##### 5.2.2.1.1 Composition

| | |
|---|---|
| Pancreatic digest of casein | 20,0 g |
| Soy lecithin | 5,0 g |
| Polysorbate 20 | 40 ml |
| Water | 960 ml |

##### 5.2.2.1.2 Preparation

Dissolve the polysorbate 20 in 960 ml of water by mixing while heating in a water bath at 49 °C ± 2 °C. Add pancreatic digest of casein and soy lecithin. Heat for about 30 min to effect solution. Mix and dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 min. After sterilization, the pH shall be equivalent to 7,3 ± 0,2 when measured at room temperature.

#### 5.2.2.2 Other neutralizing diluents

Other neutralizing diluents may be used as appropriate (see Annex A and Annex D).

### 5.2.3 Diluent

#### 5.2.3.1 Fluid A

##### 5.2.3.1.1 Composition

| | |
|---|---|
| Peptic digest of animal tissue | 1,0 g |
| Water | 1 000 ml |