



**SLOVENSKI STANDARD**  
**SIST-TS CEN/TS 17708:2023**

**01-februar-2023**

---

**Rastlinski biostimulanti - Priprava vzorcev za mikrobiološko analizo**

Plant biostimulants - Preparation of sample for microbial analysis

Pflanzen-Biostimulanzien - Probenvorbereitung für die mikrobielle Analyse

Biostimulants des végétaux - Préparation de l'échantillon pour l'analyse microbienne

**Ta slovenski standard je istoveten z: CEN/TS 17708:2022**

<https://standards.iteh.ai/catalog/standards/sist/ccc98585-1b60-4864-82a7-b4f8883088e8/sist-ts-cen-ts-17708-2023>

**ICS:**

65.080                      Gnojila                                      Fertilizers

**SIST-TS CEN/TS 17708:2023                                      en,fr,de**



TECHNICAL SPECIFICATION  
SPÉCIFICATION TECHNIQUE  
TECHNISCHE SPEZIFIKATION

**CEN/TS 17708**

March 2022

ICS 65.080

English Version

**Plant biostimulants - Preparation of sample for microbial  
analysis**

Biostimulants des végétaux - Préparation de  
l'échantillon pour l'analyse microbienne

Biostimulanzien für die pflanzliche Anwendung -  
Probenvorbereitung für die mikrobielle Analyse

This Technical Specification (CEN/TS) was approved by CEN on 3 January 2022 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

[SIST-TS CEN/TS 17708:2023](https://standards.iteh.ai/catalog/standards/sist/ccc98585-1b60-4864-82a7-b4f8883088e8/sist-ts-cen-ts-17708-2023)

[https://standards.iteh.ai/catalog/standards/sist/ccc98585-1b60-4864-82a7-  
b4f8883088e8/sist-ts-cen-ts-17708-2023](https://standards.iteh.ai/catalog/standards/sist/ccc98585-1b60-4864-82a7-b4f8883088e8/sist-ts-cen-ts-17708-2023)



EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

**CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels**

<b>Contents</b>	<b>Page</b>
European foreword.....	3
Introduction .....	4
1 Scope .....	6
2 Normative references .....	6
3 Terms and definitions .....	6
4 Principle .....	7
5 Diluents.....	7
6 Apparatus.....	7
7 Sampling and storage.....	8
8 Preparation of samples and suspension.....	8
8.1 Protocol for enumeration of beneficial microorganisms .....	8
8.2 Protocol for enumeration of not-desirable microorganisms.....	10
8.3 Protocol for the determination of anaerobic plate count.....	11
8.4 Protocol for the detection of pathogenic microorganisms .....	11
8.5 Further dilutions .....	12
8.6 Duration of the procedure .....	12
Annex A (informative) Plating procedure .....	13
Annex B (informative) Diluents for general use .....	15
Bibliography.....	17

## European foreword

This document (CEN/TS 17708:2022) has been prepared by Technical Committee CEN/TC 455 “Plant biostimulants”, the secretariat of which is held by AFNOR.

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 has been prepared under a Standardization Request given to CEN by the European Commission and the European Free Trade Association.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

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

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

[SIST-TS CEN/TS 17708:2023](https://standards.iteh.ai/catalog/standards/sist/ccc98585-1b60-4864-82a7-b4f8883088e8/sist-ts-cen-ts-17708-2023)

<https://standards.iteh.ai/catalog/standards/sist/ccc98585-1b60-4864-82a7-b4f8883088e8/sist-ts-cen-ts-17708-2023>

## Introduction

This document was prepared by the experts of CEN/TC 455 “Plant Biostimulants”. The European Committee for Standardization (CEN) was requested by the European Commission (EC) to draft European standards or European standardization deliverables to support the implementation of Regulation (EU) 2019/1009 of 5 June 2019 laying down rules on the making available on the market of EU fertilizing products (“FPR” or “Fertilising Products Regulation”).

This standardization request, presented as M/564, also contributes to the Communication on “Innovating for Sustainable Growth: A Bio economy for Europe”. The Working Group 5 “Labelling and denominations”, was created to develop a work program as part of this request. The technical committee CEN/TC 455 “Plant Biostimulants” was established to carry out the work program that will prepare a series of standards. The interest in biostimulants has increased significantly in Europe as a valuable tool to use in agriculture. Standardization was identified as having an important role in order to promote the use of biostimulants. The work of CEN/TC 455 seeks to improve the reliability of the supply chain, thereby improving the confidence of farmers, industry, and consumers in biostimulants, and will promote and support commercialisation of the European biostimulant industry.

Biostimulants used in agriculture can be applied in multiple ways: on soil, on plant, as seed treatment, etc. A microbial plant biostimulant consists of a microorganism or a consortium of microorganisms, as referred to in Component Material Category 7 of Annex II of the EU Fertilising Products Regulation.

This document is applicable to all microbial biostimulants in agriculture.

The Table 1 below summarizes many of the agro-ecological principles and the role played by biostimulants.

**Table 1 — Agro-ecological principles and the role played by biostimulants [1]**

<b>Increase biodiversity</b>
By improving soil microorganism quality/quantity
<b>Reinforce biological regulation and interactions</b>
By reinforcing plant-microorganism interactions
- symbiotic exchanges i.e. <i>Mycorrhizae</i>
- symbiotic exchanges i.e. <i>Rhizobiaceae/Faba</i>
- secretions mimicking plant hormones (i.e. <i>Trichoderma</i> )
By regulating plant physiological processes
- for e.g. growth, metabolism, plant development...
<b>Improve biogeochemical cycles</b>
- improve absorption of nutritional elements
- improve bioavailability of nutritional elements in the soil
- stimulate degradation of organic matter

This document defines the general rules for the preparation of samples, initial suspensions and subsequent dilutions for microbiological examination of plant biostimulants.

Any special diluents or practices required in specific standard methods take priority over the general rules listed in this document.

**WARNING** — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of 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.

**IMPORTANT** — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably trained staff.

## iTeh STANDARD PREVIEW (standards.iteh.ai)

[SIST-TS CEN/TS 17708:2023](https://standards.iteh.ai/catalog/standards/sist/ccc98585-1b60-4864-82a7-b4f8883088e8/sist-ts-cen-ts-17708-2023)

<https://standards.iteh.ai/catalog/standards/sist/ccc98585-1b60-4864-82a7-b4f8883088e8/sist-ts-cen-ts-17708-2023>

**CEN/TS 17708:2022 (E)****1 Scope**

This document defines general rules for the aerobic preparation of the initial suspension and of dilutions for microbiological examinations of microbial plant biostimulants.

This horizontal method might not be appropriate in very detail for certain products. In this case, different methods which are specific to these products can be used if necessary, for justified technical reasons.

**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.

CEN/TS 17702-1, *Plant biostimulants — Sampling and sample preparation — Part 1: Sampling*

CEN/TS 17724, *Plant biostimulants — Terminology*

**3 Terms and definitions**

For the purposes of this document, the terms and definitions given in CEN/TS 17724 and the following apply.

**3.1****laboratory sample**

sample sent to the laboratory and intended for inspection or testing

**3.2****test sample**

sample prepared from the laboratory sample (3.1) according to the procedure specified in the method of test and from which test portions (3.3) are taken

**3.3****test portion**

measured (volume or mass) representative sample taken from the laboratory sample (3.1) for use in the preparation of the initial suspension (3.4)

**3.4****initial suspension**

suspension, solution or emulsion obtained after a weighed or measured quantity of the product under examination has been mixed with, normally, a nine-fold quantity of diluent, or other ratios if required for specific purposes, allowing large particles, if present, to settle

**3.5****further dilution**

suspension or solution obtained by mixing a measured volume of the initial suspension (3.4) with an  $x$ -fold volume of diluent and by repeating this operation with further dilutions until a dilution series, suitable for the inoculation of culture media, is obtained

Note 1 to entry: Ten-fold dilutions are normally used to produce a decimal dilution series, but other ratios can be required for specific purposes.



### 3.6

#### **specific standard**

standard or guidance document describing the examination of a specific product for the detection or enumeration of a specific microorganism

## 4 Principle

Preparation of initial suspension (3.4) in such a way as obtain as uniform a distribution as possible of the microorganisms contained in the test portion (3.3).

Preparation, if necessary, of further dilutions (3.5) in order to reduce the number of microorganisms per unit volume to allow, after incubation, observation of their growth or not (in case of tubes or bottles) or colony counting (in the case of plates).

## 5 Diluents

For the preparation of diluents follow the current laboratory practices and the manufacturer's instructions for commercial preparations.

In Annex B there are examples of general use diluents.

It is possible to add to diluents surface-active agents to improve the uniformity of the suspension while avoiding the formation of clusters of microorganisms, and verifying that at the added concentration does not alter cell viability.

## 6 Apparatus

Usual microbiological laboratory equipment as listed below:

- 6.1 **Apparatus** for dry sterilization (oven) and wet sterilization (autoclave).
- 6.2 **Protective cabinets**, for all work involving the handling of microbiological products.
- 6.3 **Balances**, capable of weighing to 1 %of the mass.
- 6.4 **Incubator**, capable of operating at the required temperatures of the test.
- 6.5 **Total delivery pipettes**, sterile, of nominal capacity 1 ml, and graduated in divisions of 0,1 ml.
- 6.6 **Water bath**, or similar apparatus, to maintain a specific temperature.
- 6.7 **pH meter**, accurate to 0,1 pH units at 25 °C.
- 6.8 **Bottles, flasks and tubes**, for boiling and storage of culture media, and for making of dilutions.
- 6.9 **Petri dishes**, sterile, of glass or plastic, with a diameter 90 mm to 100 mm.
- 6.10 **Optical Microscope**, for bacteriological examinations, objectives with magnification from x10 (dry lens) to about x100 (oil immersion).
- 6.11 **Spreaders**, made of glass or plastic (of diameter less than 2 mm and length 80 mm). Diameter should not exceed 2 mm in order to minimize the amount of samples adhering to them at the end of the spreading procedure.

**CEN/TS 17708:2022 (E)**

**6.12 Binocular magnifier**, for discriminating and differentiation colonies/cells of yeasts and moulds (magnification 6,5 to 50 times).

**6.13 Refrigerator**, chambers which allow maintenance of cold storage.

**6.14 Centrifuge**, to separate suspended particles from fluids.

For the preparation of the test sample, of the initial suspension or for the decimal dilutions series, use one or more of the common apparatus reported below:

**6.15 Peristaltic blender (stomacher)** with sterile bags.

**6.16 Rotatory homogenizer (blender)**, with a sterilizable glass or metals bowls equipped with covers (speed between 8 000 r/min and 45 000 r/min).

**6.17 Vibrational mixer (pulsifier)** with sterile bags (usual operating time is 0,5 min to 1 min).

**6.18 Ultraturrax**, a dispersing tool and homogenizer instrumentation that produces homogenous, uniform sample preparations.

**6.19 Ultrasonic sonicator.**

**6.20 Mechanical stirrer.**

**6.21 Magnetic stirrer.**

**6.22 Vortex mixer**, mainly used for liquid samples and decimal dilutions preparation.

**6.23 Glass beads** can be used for the preparation, by shaking, of the initial suspension of certain viscous or thick products.

## 7 Sampling and storage

Carry out sampling in accordance with CEN/TS 17702-1. It is important that the laboratory receives a sample which is representative and has not been damaged or changed during transport or storage.

The samples should be delivered to the laboratory with the original storage conditions maintained as nearly as possible.

The sample should be submitted in the original, unopened container.

Upon arrival, check the condition of the samples. Identify samples clearly and completely and record the sample information.

The mode of transportation of the samples to the laboratory and the storage of the samples awaiting examination, shall ensure that they are kept under conditions which minimize any alteration in the number of microorganisms present.

## 8 Preparation of samples and suspensions

### 8.1 Protocol for enumeration of beneficial microorganisms

#### 8.1.1 General

A representative sample of the product will be prepared according to the following procedure which takes into consideration the different formulations of biostimulant based products.