



SLOVENSKI STANDARD
kSIST-TS FprCEN/TS 17710:2021
01-november-2021

[Not translated]

Plant biostimulants - Detection of *Listeria monocytogenes*

Biostimulanzien für die pflanzliche Anwendung - Nachweis von *Listeria monocytogenes*

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Ta slovenski standard je istoveten z: FprCEN/TS 17710

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Plant biostimulants - Detection of *Listeria monocytogenes*

Biostimulanzien für die pflanzliche Anwendung -
Nachweis von *Listeria monocytogenes*

This draft Technical Specification is submitted to CEN members for Vote. It has been drawn up by the Technical Committee CEN/TC 455.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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European foreword

This document (FprCEN/TS 17710:2021) has been prepared by Technical Committee CEN/TC 455 “Plant Biostimulants”, the secretariat of which is held by AFNOR.

This document is currently submitted to the vote.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EU Directive(s).

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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 fertilising products ("FPR" or "Fertilising Products Regulation"). This request, presented as SR 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 Fertilizing 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

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

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.

1 Scope

This document provides a method for the detection of *Listeria monocytogenes* in microbial plant biostimulants for verifying that the concentration of this human pathogen does not exceed the respective limits outlined in the EU Regulation on Fertilising Products [1].

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.

FprCEN/TS 17724, *Plant biostimulants — Terminology*

FprCEN/TS 17708, *Plant biostimulants — Preparation of sample for microbial analysis*

EN ISO 11290-1:2017, *Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 1: Detection method (ISO 11290-1:2017)*

EN ISO 11133:2014,¹ *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

EN ISO 7218:2007, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations (ISO 7218:2007)*

EN ISO 6887-1:2017, *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 6887-1:2017)*

3 Terms and definitions

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

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

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

3.1

listeria monocytogenes

microorganisms which form typical colonies on solid selective media described and which display the morphological, physiological and biochemical characteristics described when the analysis is carried out in accordance with this document

[SOURCE: EN ISO 11290-1:2017, 3.1]

¹ As impacted by EN ISO 11133:2014/A1:2018 and EN ISO 11133:2014/A2:2020.

FprCEN/TS 17710:2021 (E)**4 Principle****4.1 General**

The detection of *Listeria monocytogenes* requires four successive stages as specified in Annex A.

NOTE *L. monocytogenes* can be present in small numbers and is often accompanied by considerably larger numbers of bacteria belonging to different taxonomic groups or different *Listeria* species. Pre-enrichment is used to permit the detection of low numbers of *L. monocytogenes* or injured *L. monocytogenes*.

4.2 Pre-enrichment in non-selective liquid medium

Half-Fraser broth (225 ml) at ambient temperature is inoculated with the test portion sample (25 g or 25 ml), then incubated at $30\text{ °C} \pm 1\text{ °C}$ for 24-26 h.

For large quantities (e.g. 1-L or more), it is recommended to pre-warm the broth to 30 °C before mixing it with the test portion.

4.3 Enrichment in/on selective media

Fraser broth is inoculated at 37 °C (0,1 ml of culture in 10 ml of Fraser broth) and incubated at $37\text{ °C} \pm 1\text{ °C}$ for $24\text{ h} \pm 2\text{ h}$.

4.4 Plating out on selective solid media

Streak both primary AND secondary enrichments onto:

- Agar *Listeria* according to Ottaviani and Agosti (ALOA) [3];
- A second selective agar of choice, e.g. PALCAM agar, Oxford agar.

The agar prepared according to Ottaviani and Agosti is incubated $24\text{ h} \pm 2\text{ h}$, $37\text{ °C} \pm 1\text{ °C}$ and additionally $24\text{ h} \pm 2\text{ h}$, $37\text{ °C} \pm 1\text{ °C}$, then examined. The second selective agar is incubated as specified by the manufacturer.

4.5 Confirmation

Colonies of presumptive *L. monocytogenes* are subcultured and their identity is confirmed by means of appropriate morphological and biochemical tests.

5 Culture media, reagents, antisera

For current laboratory practices FprCEN/TS 17708 and EN ISO 11133:2014 shall be used.

Composition of culture media and reagents and their preparation are described in Annex B.

6 Equipment and consumables

Disposable equipment is an acceptable alternative to reusable glassware if it has suitable specifications.

Usual microbiological laboratory equipment according to EN ISO 7218:2007 shall be used and, in particular, the following.

- 6.1** Apparatus for dry sterilization (oven) or wet sterilization (autoclave), as specified in EN ISO 7218:2007.
- 6.2** Drying cabinet or incubator, capable of operating between 25 °C and 50 °C .
- 6.3** Incubators, capable of operating at $30\text{ °C} \pm 1\text{ °C}$, $37\text{ °C} \pm 1\text{ °C}$, and at $25\text{ °C} \pm 1\text{ °C}$ (optional).

- 6.4 Water bath, capable of operating at 47 °C to 50 °C.
- 6.5 Sterile loops, approximately 3 mm in diameter or 10 µL, and inoculating needle or wire.
- 6.6 pH-meter, having an accuracy of calibration of ± 0,1 pH unit at 25 °C.
- 6.7 Sterile graduated pipettes or automatic pipettes of nominal capacities of 1 ml, and 10 ml.
- 6.8 Sterile Petri dishes, with a diameter of approximately 90 mm and (optional) large size (diameter approximately 140 mm).
- 6.9 Microscope, preferably with phase-contrast, and with slides and cover slips.
- 6.10 Refrigerator, capable of operating at 5 °C ± 3 °C.
- 6.11 Peristaltic blender (stomacher) with 400 ml sterile bags.
- 6.12 Blender motor and jars or vortex.

7 Sampling

Sampling is not part of the method specified in this document (see the specific European EN Standard dealing with the product concerned). If there is no specific International or European Standard, it is recommended that the parties concerned come to an agreement on this subject.

It is important that the laboratory receives a sample which is representative and has not been damaged or changed during transport or storage.

8 Preparation of test sample

Prepare the test sample from the laboratory sample in accordance with the specific International or EN Standard dealing with the product concerned [see EN ISO 6887-1:2017].

9 Preparation of test sample

9.1 Test portion and initial suspension

To ensure a truly representative analytical unit, agitate liquids or free flowing materials until the contents are homogeneous. If the sample unit is a solid, obtain the analytical unit by taking a portion from several locations within the sample unit. To reduce the workload, the analytical units may be combined for analysis. It is recommended that a composite contains no more than five analytical units.

General rules for the preparation of the initial suspension for microbiological examination are described in FprCEN/TS 17708. The initial suspension of the microbial biostimulant product samples can be prepared according to EN ISO 11290-1:2017 using, as dilution fluid, the selective primary enrichment medium, half-Fraser broth, described in B.1.

A representative sample of the product should be prepared taking into consideration the different formulations of the biostimulant-based products.

Add 25 g or 25 ml of the product (the analytical unit) to 225 ml of half-Fraser broth in a 400 ml stomacher bag (6.11) or a blender jar (6.12). For composite samples, analytical units may be combined up to 125 g or ml (e.g. 125 g or ml of food to 1 125 ml of half-Fraser broth). Pre-warm the half-Fraser broth to room temperature before use.

If alternate analytical units are required, maintain a ratio of 1 part sample material to 9 parts half-Fraser broth.