
**Soil quality — Measurement of
enzyme activity patterns in soil
samples using fluorogenic substrates
in micro-well plates**

*Qualité du sol — Mesure en microplaques de l'activité enzymatique
dans des échantillons de sol en utilisant des substrats fluorogènes*



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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).

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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 190, *Soil quality*, Subcommittee SC 4, *Biological characterization*.

This second edition cancels and replaces the first edition (ISO/TS 22939:2010), which has been technically revised. The main changes compared to the previous edition are as follows:

- [Clause 3](#) “Terms and definitions” added;
- [6.2.4](#): unit corrected in (40 ml to 40 µl);
- [6.2.6](#), [Table 1](#) (Chitinase change E.C. 3.2.1.30 to E.C.3.2.1.52 and Alanin-aminopeptidase E.C. 3.4.11.12 to E.C. 3.4.11.2).

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.

Introduction

Micro-organisms are responsible for many key processes in the cycle of elements. Enzymes play key roles in the degradation and mineralization of organic macromolecules. The main postulate is the microbial origin of soil enzymes, even if plant root exudates include enzymes. The simultaneous monitoring of several enzyme activities important in the biodegradation of organic compounds and mineralization of C, N, P and S in soil may reveal harmful effects caused by chemicals and other anthropogenic impacts (e.g. acidification, compaction). However, the measurements carried out under selected laboratory conditions using artificial substrates cannot be a substitute for the actual rate of enzymatic processes in soil in situ.

Soil quality — Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates

1 Scope

This document specifies a method for the measurement of several enzyme activities (arylsulfatase, α -glucosidase, β -glucosidase, Cellubisidase, β -Xylosidase, phosphodiesterase (PDE), chitinase, phosphomonoesterase (PME), leucine-aminopeptidase, Alanine-aminopeptidase) simultaneously (or not) using fluorogenic substrates in soil samples. Enzyme activities of soil vary seasonally and depend on the chemical, physical and biological characteristics of soil. Its application for the detection of harmful effects of toxic chemicals or other anthropogenic impacts depends on the simultaneous comparison of enzyme activities in a control soil similar to the test soil, or on exposure tests with chemicals or treatments.

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 18400-206, *Soil quality — Sampling — Part 206: Collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

ISO 10390, *Soil quality — Determination of pH*

ISO 10694, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis)*

3 Terms and definitions

No terms and definitions are listed in this document.

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 Abbreviated terms

E.C.	Enzyme code number defined by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB)
SOM	Soil organic matter content
MUB	Modified universal buffer

5 Principle

This document describes a method for the simultaneous measurements of several enzymes in soil samples. It is based on the use of soil samples diluted in buffer containing fluorogenic substrates, which