
**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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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Contents

Page

Foreword	iv
Introduction.....	v
1 Scope.....	1
2 Normative references	1
3 Abbreviated terms	1
4 Principle.....	1
5 Reagents.....	2
6 Apparatus and materials.....	5
7 Procedure	6
8 Calculation of results	7
9 Expression of results	8
10 Test report.....	8
Annex A (informative) Guidance on the use of freshly prepared substrates	9
Annex B (informative) Example of a graph for calculation.....	11
Bibliography.....	13

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.

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In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

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An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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Introduction

Micro-organisms are responsible for many key processes in the cycle of elements. Enzymes are responsible for the degradation of organic molecules and their mineralization. The main postulate is the microbial origin of soil enzymes, even if plant root exudates include enzymes. Extracellular enzymes in soil play key roles in the biodegradation of organic macromolecules. 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. 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*.

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

1 Scope

This Technical Specification specifies a method for the measurement of several enzyme activities simultaneously 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 referenced documents are indispensable for the application 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 10381-6, *Soil quality — Sampling — Part 6: Guidance on the 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 carbon and total carbon after dry combustion (elementary analysis)*

3 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

4 Principle

This Technical Specification 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 are incubated for 3 h at $(30 \pm 2)^\circ\text{C}$ in multi-well plates. After the incubation the enzyme activities are measured as fluorescence with a plate-reading fluorometer (References [1] and [2] in the Bibliography). The method described is based on dried standard and substrate plates enabling storage and limiting bias due to differences between reagent batches, and also enabling comparison between reagent batches. Annex A describes a method utilizing freshly prepared reagents, which has a clearly defined and exact incubation period. The advantage of the use of freshly prepared substrates is that an instrument for lyophilization is not required.