SLOVENSKI STANDARD
oSIST prEN ISO 20130:2020

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Kakovost tal - Merjenje encimske aktivnosti v vzorcih tal s kolorometričnimi substrati (ISO 20130:2018)

Soil quality - Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates (ISO 20130:2018)

Qualité du sol - Mesure de l'activité enzymatique dans des échantillons de sol en utilisant des substrats colorimétriques (ISO 20130:2018)

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

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

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Foreword

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This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization*.

Introduction

Microorganisms 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 carbon, nitrogen, phosphorus and sulfur 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*.

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

1 Scope

This document specifies a method for the measurement of several hydrolase activities (arylamidase, arylsulfatase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-glucosaminidase, acid, alkaline and global phosphatases, urease) simultaneously (or not) in soil samples, using colorimetric substrates. Enzyme activities of soil vary seasonally and depend on soil chemical, physical and biological characteristics. This method can be applied either to detect harmful effects on soil enzyme activities derived from toxic substances or other anthropogenic agents in contaminated soils against a control soil, or to test chemicals.

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*

3 Terms and definitions, symbols and abbreviated terms

3.1 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:

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

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

3.2 Symbols and abbreviated terms

ARN	Arylamidase
ARS	Arylsulfatase
E.C.	Enzyme code number by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB)
NAG	N-acetyl-glucosaminidase
PAC	acid phosphatase
PAK	alkaline phosphatase
PHOS	phosphatase

URE urease

β–GAL *β*-galactosidase

α–GLU *α*-glucosidase

β–GLU *β*-glucosidase

4 Principle

This document describes a method for the simultaneous measurement of several enzymes in soil samples (see Table 1). It is based on the use of soil samples solutions and colorimetric substrates, which are incubated during specific times at 25 °C ± 2 °C or 37 °C ± 2 °C in multi-well plates. After the incubation, reactions are stopped, plates are then centrifuged and supernatants transferred into new plates. The intensities of the coloration are measured with absorbance with a 96 wells microplate spectrophotometer UV/visible.

Table 1 — Enzymatic activity measurements with colorimetric method

Enzyme	Abbreviation	N°	Soil cycle	Macromolecule degraded
Arylamidase	ARN	E.C. 3.4.11.2	Nitrogen	
Arylsulfatase	ARS	E.C. 3.1.6.1	Sulfur	Mineralization of organic sulfur
β-Galactosidase	βGAL	E.C. 3.2.1.22	Carbon	Hemicellulose
α-Glucosidase	αGLU	E.C. 3.2.1.20	Carbon	Starch and glycogen
β-Glucosidase	βGLU	E.C. 3.2.1.21	Carbon	Cellulose
N-acetyl-glucosaminidase	NAG	E.C. 3.2.1.52	Carbon	Chitin and other *β*-1,4-linked glucosamine polymers
Phosphatase	PHOS	E.C. 3.1.4.1	Phosphorus	Phosphate esters
Acid phosphatase	PAC	E.C. 3.1.4.1	Phosphorus	Phosphate esters
Alkaline phosphatase	PAK	E.C. 3.1.4.1	Phosphorus	Phosphate esters
Urease	URE	E.C. 3.5.1.5	Nitrogen	Urea

An interlaboratory trial was carried out for the validation of the standard; summary of the international ring test is given in Table 8, and the whole data of the interlaboratory validation are described in Annex B.

5 Reactives

5.1 Buffers and reageants

5.1.1 General

The choice is made to use deionized water as medium to evaluate native soil enzyme activities at soil pH and also to allow the analysis of multiple enzymes using the same soil suspension. The soil (in g)/water (in ml) ratio (4:25) is optimized to maximize reaction, sensitivity and facilitate pipetting technique. The use of the same soil solution for analysing multiple enzymes also makes data more comparable. Arylamidase is measured with Tris buffer 50 mmol/l, pH 7,5 and acid and alkaline phosphatases are involved with the use of Tris-HCl 50 mmol/l at pH 5,5 and Tris base 50 mmol/l at pH 11, respectively.

NOTE The volume can be adapted according to needs.

5.1.2 Tris hydrochloride 50 mmol/l pH 5,5 ± 0,1.

— Tris(hydroxymethyl)aminomethane hydrochloride (CAS N°: 1185-53-1 – Mw:157,6): 7,88 g;

— deionized water: 1 000 ml;

— hydrochloric acid (HCl) (CAS N°7647-01-0) 1 mol/l.

Dissolve 7,88 g of Tris(hydroxymethyl)aminomethane hydrochloride into 800 ml deionized water and adjust to pH 5,5 with hydrochloric acid (1 mol/l). Fill in to 1 000 ml. The storage duration shall not exceed one month at 4 °C ± 2 °C in glass or polypropylene bottle.

5.1.3 Tris base 50 mmol/l pH 11 ± 0,1.

— Tris(hydroxymethyl)aminomethane (CAS N°: 77-86-1 - Mw:121,14): 6,06 g;

— deionized water: 1 000 ml;

— sodium hydroxide (CAS N° 1310-73-2) (1 mol/l).

Dissolve 6,06 g of Tris(hydroxymethyl)aminomethane into 800 ml deionized water and adjust to pH 11 with sodium hydroxide (1 mol/l). Fill in to 1 000 ml. The storage duration shall not exceed one month at 4 °C ± 2 °C.

5.1.4 Tris base 50 mmol/l pH 7,5 ± 0,1.

— Tris(hydroxymethyl)aminomethane (CAS N°: 77-86-1 - Mw:121,14): 6,06 g;

— deionized water: 1 000 ml;

— hydrochloric acid (HCl) (CAS N°7647-01-0) 1 mol/l.

Dissolve 6,06 g of Tris(hydroxymethyl)aminomethane into 800 ml deionized water and adjust to pH 7,5 with hydrochloric acid (1 mol/l). Fill in to 1 000 ml. The storage duration shall not exceed one month at 4 °C ± 2 °C.

5.1.5 Tris base 100 mmol/l pH 12 ± 0,1.

— Tris(hydroxymethyl)aminomethane (CAS N°: 77-86-1 - Mw:121,14): 12,11 g;

— deionized water: 1 000 ml;

— sodium hydroxide (CAS N° 1310-73-2) (5 mol/l).

Dissolve 12,11 g of Tris(hydroxymethyl)aminomethane into 800 ml deionized water and adjust to pH 12 with sodium hydroxide (5 M). Fill in to 1 000 ml. The storage duration shall not exceed one month at 4 °C ± 2 °C.

5.1.6 Calcium chloride dihydrate 0,5 mol/l.

— calcium chloride dihydrate (CAS N°: 10035-04-8 - Mw:147,01): 14,7 g;

— Deionized water: 200 ml.

Dissolve 14,7 g of calcium chloride dihydrate in 200 ml of deionized water. The storage duration shall not exceed one month at 4 °C ± 2 °C.

5.1.7 Salicylate reagent.

— sodium salicylate 270 mmol/l (CAS N°: 54-21-7 - Mw:160,1): 865 mg;

— tri sodium citrate 145 mmol/l (CAS N°: 6132-04-3 - Mw:294,1): 853 mg;

— di sodium tartrate 60 mmol/l (CAS N°: 6106-24-7 - Mw:230,08): 276 mg;

— sodium nitroferricyanide 2 mmol/l (CAS N°: 13755-38-9 - Mw:297,95): 12 mg;

— deionized water: 20 ml.

Salicylate reagent is prepared with the 4 compounds listed above just before analysis; dissolve 865 mg of sodium salicylate, 853 mg of tri sodium citrate, 276 mg of di sodium tartrate and 12 mg of sodium nitroferricyanide in 20 ml of deionized water.

5.1.8 Cyanurate reagent.

— tri sodium citrate 580 mmol/l (CAS N°: 6132-04-3 - Mw:294,1): 3,4 g;

— di sodium tartrate 90 mmol/l (CAS N°: 6106-24-7 - Mw:230,08): 414 mg;

— lithium hydroxide 280 mmol/l (CAS N° : 1310-65-2 - Mw:23,95): 134 mg;

— dichloroisocyanurate 10 mmol/l (CAS N° : 51580-86-0 - Mw:255,98): 51 mg;

— deionized water: 20 ml.

Cyanurate reagent is prepared with the 4 compounds listed above just before analysis; dissolve 3,4 g of tri sodium citrate, 414 mg of di sodium tartrate, 134 mg of lithium hydroxide and 51 mg of dichloroisocyanurate in 20 ml of deionized water.

5.1.9 Ethanol, 96 %.

— Ethanol 96 % (CAS N° 41340-36-7).

5.1.10 Acidified ethanol (0,26 mol/l HCl).

— Hydrochloric acid ACS reagent, 37 % (CAS N°7647-01-0) 4,32 ml;

— Ethanol 96 % (CAS N° 41340-36-7).

Dilute 4,32 mL of concentrated HCl into 200 ml ethanol 96 %. The storage duration shall not exceed one month at 4 °C ± 2 °C.

5.1.11 p-dimethylaminocinnamaldehyde (DMCA) (3,5 mmol/l).

— DMCA (CAS N°: 6203-18-5 - Mw:175,23): 0,12 g;

— Ethanol 96 % (CAS N° 41340-36-7).

Dissolve 0,12 g of DMCA into 200 ml ethanol 96 %. The storage duration shall not exceed one week at –20 °C ± 2 °C.

Table 2 — Buffer utilization for enzymatic activity measurement

	ARS; α-GLU; β-GLU; β-GAL; NAG; PHOS;	ARN	URE	PAC	PAK
Soil solution	deionized water	Tris base 50 mmol/l, pH 7,5	deionized water	Tris HCl 50 mmol/l, pH 5,5	Trisbase 50 mmol/l, pH 11
Stop/ revelation	Tris 100 mmol/l pH12	Ethanol 96 % Acidified ethanol	salicylate reagent	Tris 100 mmol/l pH 12	
	$CaCl_2$ 0,5 mol/l	DMCA	cyanurate reagent	$CaCl_2$ 0,5 mol/l	