

---

---

**Soil quality — Determination of  
dehydrogenase activity in soils —**

**Part 1:  
Method using triphenyltetrazolium  
chloride (TTC)**

*Qualité du sol — Détermination de l'activité des déshydrogénases dans  
les sols —*

*Partie 1: Méthode au chlorure de triphényltétrazolium (CTT)*



Reference number  
ISO 23753-1:2005(E)

© ISO 2005

**PDF disclaimer**

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. The ISO Central Secretariat accepts no liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies. In the unlikely event that a problem relating to it is found, please inform the Central Secretariat at the address given below.

© ISO 2005

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from either ISO at the address below or ISO's member body in the country of the requester.

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](mailto:copyright@iso.org)  
Web [www.iso.org](http://www.iso.org)

Published in Switzerland

# Contents

Page

<b>Foreword</b> .....	<b>iv</b>
<b>Introduction</b> .....	<b>v</b>
<b>1 Scope</b> .....	<b>1</b>
<b>2 Normative references</b> .....	<b>1</b>
<b>3 Principle</b> .....	<b>1</b>
<b>4 Reagents and materials</b> .....	<b>2</b>
<b>5 Apparatus</b> .....	<b>3</b>
<b>6 Procedure</b> .....	<b>3</b>
<b>7 Calculation</b> .....	<b>3</b>
<b>8 Test report</b> .....	<b>4</b>
<b>Bibliography</b> .....	<b>5</b>

## 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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 23753-1 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

ISO 23753 consists of the following parts, under the general title *Soil quality — Determination of dehydrogenase activity in soils*:

- *Part 1: Method using triphenyltetrazolium chloride (TTC)*
- *Part 2: Method using iodotetrazolium chloride (INT)*

## Introduction

The soil microflora is responsible for the decomposition and conversion of organic substances, aggregation stability and the carbon, nitrogen, sulfur and phosphorus cycles. Dehydrogenases, as respiratory chain enzymes, play a major role in the energy production by organisms. They oxidize organic compounds by transferring two hydrogen atoms. Dehydrogenases are essential components of the enzyme system of microorganisms. Dehydrogenase activity can therefore be used as an indicator of biological redox systems and as a measure of microbial activity in the soil.

Copyright International Organization for Standardization

# Soil quality — Determination of dehydrogenase activity in soils —

## Part 1: Method using triphenyltetrazolium chloride (TTC)

### 1 Scope

This part of ISO 23753 specifies a method for determining the dehydrogenase activity in soil using 2,3,5-triphenyltetrazolium chloride (TTC).

It is not applicable for determining the dehydrogenase activity in the upper layers (L, F, H horizons) of forest humus forms with low microbial activity (e.g. mor), or in soils showing reducing properties (e.g. waterlogged soils).

### 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 for the assessment of aerobic microbial processes in the laboratory*

ISO 10390, *Soil quality — Determination of pH*

ISO 11259, *Soil quality — Simplified soil description*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

### 3 Principle

TTC solution is added to a soil sample and the mixture is incubated at 25 °C for 16 h. The triphenylformazan (TPF) released is extracted with acetone and determined by photometry at a wavelength of 485 nm.

NOTE 1 The method is based on a modified version of the method reported in Reference [3].

NOTE 2 Other extraction liquids than acetone may be used (e.g. ethanol, acetone-CCl<sub>4</sub> mixture). According to Reference [1], methanol is not suitable because the extraction efficiency is influenced by the soil and the soil water content.

In the case of soil having reducing characteristics (e.g. waterlogged soil), dehydrogenase activity should not be used as a measure of the biological activity in the soil [2]. Abiotic components, such as iron(II) compounds or sulfides, can reduce TTC.

NOTE 3 Organic solvents can extract excessive amounts of humic substances from humus rich soils (e.g. L, F, H horizons, mouldy peat), giving blank values ( $X_{LP}$ ) which do not differ from measured values ( $X_{VP}$ ) in soils with low microbial activity.

## 4 Reagents and materials

### 4.1 Soil

Take and prepare soil samples as specified in ISO 10381-6. If samples which have been sieved in the fresh state cannot be analysed immediately, they may be kept for up to three months at 4 °C. Determine the dry matter content of the sample in accordance with ISO 11465.

The storage can affect the enzyme activity and hence dehydrogenase activity of samples with different storage times should not be compared.

**4.2 Hydrochloric acid**,  $c(\text{HCl}) = 1 \text{ mol/l}$ .

**4.3 Tris buffer solution**,  $c = 0,1 \text{ mol/l}$ .

Dissolve 12,11 g of tris(hydroxymethyl)aminomethane in 600 ml of distilled water and adjust the pH value to

- a) 7,8 for acidic soil (pH less than 6);
- b) 7,6 for neutral soil (pH 6 to 7);
- c) 7,4 for carbonate-rich soil (pH greater than 7);

using 1 mol/l hydrochloric acid and make up to 1 000 ml with distilled water. Determine pH value in accordance with ISO 10390.

### 4.4 Substrate solution (TTC)

The optimum substrate concentration is generally between 0,1 % and 2 % TTC, depending on soil textural class and humus content. Determine the soil texture in accordance with ISO 11259.

Dissolve 2,3,5-triphenyltetrazolium chloride in tris buffer solution (4.3). The solution may be stored for one week at 4 °C in the dark.

Depending on the humus and clay content of the sample, use the following substrate concentrations:

- a) sandy soil, slightly humic and slightly clayey soil: 0,1 % to 0,5 % TTC;
- b) loam, humic and loamy soil: 0,6 % to 1,0 % TTC;
- c) clayey and humic soil: 1,0 % to 2,0 % TTC.

### 4.5 Analytical grade acetone

### 4.6 Triphenylformazan (TPF) solutions

**4.6.1 TPF stock solution**, dissolve 1 000 mg of triphenylformazan (TPF) in acetone (4.5) to make up 100 ml.

**4.6.2 TPF working solution**, dilute 1,0 ml of stock solution (4.6.1) to 100 ml with acetone (4.5).

#### 4.6.3 TPF calibration solutions

Pipette 0 ml, 1,0 ml, 2,0 ml, 5,0 ml and 10,0 ml respectively, of the solution specified in 4.6.2 into a series of five test tubes and make the volume up to 30 ml with acetone (4.5). The solutions obtained have TPF concentrations of 0 µg/ml, 3,33 µg/ml, 6,67 µg/ml, 16,7 µg/ml and 33,3 µg/ml respectively.

Since TTC and TPF are light-sensitive, the solutions should be protected from exposure to light throughout the analysis.



## 5 Apparatus

### 5.1 Photometer

### 5.2 pH meter

### 5.3 Test tubes

For example, 2 cm diameter and at least 30 ml capacity (test portion 5 g, see also Clause 6).

### 5.4 Incubator, capable of being set to $(25 \pm 1) ^\circ\text{C}$ .

### 5.5 Suitable conical flasks, volumetric flasks, pipettes and funnels

### 5.6 Test tube shaker

### 5.7 Fluted filter, with slow filtering action (90 s to 100 s).

## 6 Procedure

Weigh 5,00 g portions of naturally moist soil into each of four test tubes (5.3). Add 5 ml of substrate solution (4.4) to three samples (complete samples). Pipette 5 ml of tris buffer solution (4.3) instead of the substrate solution to the blank sample. Shake each tube, seal the tubes with rubber stoppers and incubate them at  $25 ^\circ\text{C}$  for 16 h. To extract the triphenylformazan formed, add 25 ml of acetone (4.5) to the samples and allow them to stand for 2 h in the dark. Shake the samples after adding the extractant, after 1 h, and again after 2 h. Then filter the samples in semidarkness using a fluted filter.

Within 1 h, measure the absorbance of the filtrates against the calibration curve zero by photometry at a wavelength of 485 nm.

According to Reference [1], optimum conditions are given at a soil/solution ratio of 1:1. There is no linear correlation between test portion and TTC reduction if the same test tubes are used. If the test tube diameter is fitted to the test portion, a linear correlation between TTC reduction and the mass of test portion is obtained. Optimum test tube diameters are 1,5 cm for 2 g of soil, 2 cm for 5 g of soil and 2,5 cm for 10 g of soil.

For L, F, H horizons, soil portions of 0,5 g or 1 g and a test tube of 2 cm diameter are recommended [4].

## 7 Calculation

Determine the dehydrogenase activity (based on dry soil) from the calibration curve and the following equation:

$$a = \frac{(\bar{\rho}_{\text{cs}} - \bar{\rho}_{\text{bs}}) \times V \times 100}{m \times \text{DM} \times t}$$

where:

$a$  is the dehydrogenase activity in  $\mu\text{g/g}$  of dry soil per hour;

$\bar{\rho}_{\text{cs}}$  is the complete sample average TPF concentration, in  $\mu\text{g/ml}$ ;

$\bar{\rho}_{\text{bs}}$  is the average TPF concentration of the blank sample, in  $\mu\text{g/ml}$ ;

$V$  is the solution volume (= volume of substrate or buffer solution + volume of extractant), in ml;

## ISO 23753-1:2005(E)

$m$  is the initial soil sample mass, in g;

DM is the sample dry matter content in accordance with ISO 11465, as a percentage;

$t$  time of incubation (here 16 h), in h.

### 8 Test report

The test report shall refer to this part of ISO 23753 and include the following details:

- a) sample identification;
- b) details of storage temperature and duration (4.1);
- c) soil textural class;
- d) pH value (pH CaCl<sub>2</sub>) of soil sample;
- e) test results;
- f) any details that are optional or deviations from the specifications of this part of ISO 23753, and any effects which may have affected the results.

## Bibliography

- [1] THALMANN, H.: Zur Methodik der Bestimmung der Dehydrogenaseaktivität im Boden mittels Triphenyltetrazoliumchlorid (TTC). *Landwirtsch. Forsch.* **21**, 1968, pp. 243–258
- [2] GLATHE, H. and THALMANN, H.: Microbial activity and its relationship to fertility characteristics of some agricultural soils, with particular reference to dehydrogenase activity. Part 2. *Zbl Bakt II*, **124**, 1970, pp. 24–36
- [3] ÖHLINGER, R.: Determination of dehydrogenase activity using TTC. In: *Methods in soil biology*. (SCHINNER, F., ÖHLINGER, R., KANDELER, E. and MARGESIN, R. eds.). Springer-Verlag, Berlin/Heidelberg/New York, 2nd edn., 1993
- [4] WILKE, B.-M.: Über Sorption und Wirkung von Blei auf die biologische Aktivität terrestrischer Humusformen. *Z. Pflanzenernaehr. Bodenk.* **145**, 1982, pp. 52–65

---

---

**ICS 13.080.30**

Price based on 5 pages