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**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)*



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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.

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.

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# 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.