

# ILNAS

Institut luxembourgeois de la normalisation  
de l'accréditation, de la sécurité et qualité  
des produits et services

## ILNAS-EN ISO 21286:2020

### **Soil quality - Identification of ecotoxicological test species by DNA barcoding (ISO 21286:2019)**

Qualité du sol - Identification des espèces  
par codes-barres ADN dans les essais  
d'écotoxicologie (ISO 21286:2019)

Bodenbeschaffenheit - Identifizierung  
der Testorganismenarten für  
ökotoxikologische Tests mit Hilfe von  
DNA-Barcoding (ISO 21286:2019)

## National Foreword

This European Standard EN ISO 21286:2020 was adopted as Luxembourgish Standard ILNAS-EN ISO 21286:2020.

Every interested party, which is member of an organization based in Luxembourg, can participate for FREE in the development of Luxembourgish (ILNAS), European (CEN, CENELEC) and International (ISO, IEC) standards:

- Participate in the design of standards
- Foresee future developments
- Participate in technical committee meetings

<https://portail-qualite.public.lu/fr/normes-normalisation/participer-normalisation.html>

### **THIS PUBLICATION IS COPYRIGHT PROTECTED**

Nothing from this publication may be reproduced or utilized in any form or by any mean - electronic, mechanical, photocopying or any other data carries without prior permission!

EUROPEAN STANDARD <sup>ILNAS-EN ISO 21286:2020</sup>  
NORME EUROPÉENNE  
EUROPÄISCHE NORM

April 2020

ICS 13.080.30

English Version

**Soil quality - Identification of ecotoxicological test species  
by DNA barcoding (ISO 21286:2019)**

Qualité du sol - Identification des espèces par codes-barres ADN dans les essais d'écotoxicologie (ISO 21286:2019)

Bodenbeschaffenheit - Allgemeine Anleitung zur Verwendung des DNA-Barcodes in ökotoxikologischen Untersuchungen (ISO 21286:2019)

This European Standard was approved by CEN on 13 April 2020.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

**CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels**

## Contents

	Page
European foreword.....	3

## European foreword

The text of ISO 21286:2019 has been prepared by Technical Committee ISO/TC 190 "Soil quality" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 21286:2020 by Technical Committee CEN/TC 444 "Environmental characterization of solid matrices" the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2020, and conflicting national standards shall be withdrawn at the latest by October 2020.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

## Endorsement notice

The text of ISO 21286:2019 has been approved by CEN as EN ISO 21286:2020 without any modification.

First edition  
2019-03

---

---

---

## **Soil quality — Identification of ecotoxicological test species by DNA barcoding**

*Qualité du sol — Identification des espèces par code-barre ADN dans  
les essais d'écotoxicologie*



**COPYRIGHT PROTECTED DOCUMENT**

© ISO 2019

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office  
CP 401 • Ch. de Blandonnet 8  
CH-1214 Vernier, Geneva  
Phone: +41 22 749 01 11  
Fax: +41 22 749 09 47  
Email: [copyright@iso.org](mailto:copyright@iso.org)  
Website: [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 Terms and definitions</b>	<b>1</b>
<b>4 Principle</b>	<b>2</b>
<b>5 Reagents and material</b>	<b>3</b>
5.1 Biological material	3
5.2 Enzyme	3
5.3 Oligonucleotide PCR primers	3
5.4 Reagents	3
<b>6 Apparatus</b>	<b>4</b>
<b>7 General requirements</b>	<b>5</b>
7.1 Experimental precaution and contamination avoidance	5
7.2 Safety precautions	5
7.2.1 Chemical hazards	5
7.2.2 Physical hazards	6
<b>8 Procedure</b>	<b>6</b>
8.1 DNA isolation	6
8.2 Quantification	7
8.3 PCR	7
8.3.1 Target genomic region	7
8.3.2 Primer design	7
8.3.3 Primer synthesis	7
8.3.4 PCR	8
8.4 Checking the amplicon size	9
8.5 Purification	9
8.6 Sequencing	9
8.7 Bioinformatics	9
8.7.1 General	9
8.7.2 Electropherogram or raw sequence quality checking	10
8.7.3 Trimming of low-quality regions and primers sequences	10
8.7.4 Sequence overlapping	10
8.7.5 Sequence verification	11
8.7.6 Reviewing the edited sequence	11
8.7.7 Species assignment	11
8.7.8 Quality of the reference databases	12
<b>9 Calculation and expression of results</b>	<b>13</b>
<b>10 Validity of the test</b>	<b>13</b>
<b>11 Test report</b>	<b>14</b>
<b>Annex A (informative) <i>Eisenia</i> Barcoding Initiative: A ring test to evaluate the applicability of DNA barcoding for the identification of <i>Eisenia</i> species</b>	<b>15</b>
<b>Bibliography</b>	<b>18</b>