

ISO 17601:2025-10 (E)

Soil quality - Estimation of abundance of selected microbial gene sequences by quantitative polymerase chain reaction (qPCR) from DNA directly extracted from soil

Contents		Page
Foreword		iv
Introduction		v
1	Scope	1
2	Normative references	1
3	Terms and definitions	1
4	Principle	2
5	Test materials	4
5.1	DNA	4
5.2	Bacteria	4
5.3	Plasmid	4
5.4	Enzymes	4
5.5	Chemicals	4
5.6	Products for bacterial culture medium	5
5.7	Buffers and reagents	5
6	Apparatus	6
7	Procedure	6
7.1	qPCR standard preparation and calibration of qPCR assay (task 1)	6
7.1.1	General	6
7.1.2	Amplicon design (task 1, step 1)	6
7.1.3	qPCR standard preparation (task 1, step 2)	7
7.1.4	Bacterial isolate DNA, environmental DNA, artificial DNA	7
7.1.5	Calibration of the qPCR (task 1, step 3)	9
7.2	Preparation of soil DNA template and inhibition test (task 2)	10
7.2.1	General	10
7.2.2	Soil DNA preparation (task 2, step 4)	10
7.2.3	Inhibition test (task 2, step 5)	10
7.3	qPCR assay (task 3)	12
7.3.1	General	12
7.3.2	qPCR (task 3, step 6)	12
7.4	Validation and analysis of qPCR assay (task 4)	12
7.4.1	General	12
7.4.2	Validation of the qPCR assay (task 4, step 7)	12
7.4.3	Calculation of the copy number of the gene of interest in the soil DNA extract (task 4, step 8)	13
8	Examination of the critical steps of the qPCR assay	13
9	Expression of the results of the qPCR assay	14
10	International ring test	14
11	Test report	14
Annex A (informative)	Description of principal steps of TaqMan® qPCR assay	15
Annex B (informative)	International ring test for evaluating qPCR to quantify the abundance of selected microbial gene sequences from DNA directly extracted from soil	17
Annex C (informative)	Examples of well-established primer systems for a qPCR-based quantification of marker genes in soil samples	30
Bibliography		33