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Water quality — Determination of the estrogenic potential of water and waste water —

Part 2: Yeast estrogen screen (A-YES, *Arxula adeninivorans*)

Qualité de l'eau — Détermination du potentiel oestrogène de l'eau et des eaux résiduaires —

Partie 2: Test d'oestrogénicité (A-YES, Arxula adeninivorans*)*

Reference number
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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

A list of all parts in the ISO 19040 series can be found on the ISO website.

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Water quality — Determination of the estrogenic potential of water and waste water —

Part 2:
Yeast estrogen screen (A-YES, *Arxula adeninivorans*)

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably trained staff.

1 Scope

This document specifies a method for the determination of the estrogenic potential of water and waste water by means of a reporter gene assay with a genetically modified yeast strain *Arxula adeninivorans*. This reporter gene assay is based on the activation of the human estrogen receptor alpha.

Arxula adeninivorans is a highly robust and salt- and temperature-tolerant test organism and is especially suitable for the analysis of samples with high salinity (conductivity up to 70 mS/cm). The test organism can be cultivated in medium with sodium chloride content up to 20 %.

This method is applicable to:

— fresh water;

— waste water;

— sea water;

— brackish water;

— aqueous extracts and leachates;

— eluates of sediments (fresh water);

— pore water;

— aqueous solutions of single substances or of chemical mixtures;

— drinking water.

The limit of quantification (LOQ) of this method for the direct analysis of water samples is between 1,5 ng/l and 3 ng/l 17β-estradiol equivalents (EEQ). The upper threshold of the dynamic range for this test is between 25 ng/l and 40 ng/l 17β-estradiol equivalents (EEQ). Samples showing estrogenic potencies above this threshold have to be diluted for a valid quantification. Extraction and pre-concentration of water samples can prove necessary, if their estrogenic potential is below the given LOQ.

An international interlaboratory trial for the validation of this document has been carried out. The results are summarized in Annex F.

NOTE Extraction and pre-concentration of water samples can prove necessary.

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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 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

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

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

3.1
blank replicate
additional replicate that contains no test organism, but is treated in the same way as the other replicates of a sample

[SOURCE: ISO 10872:2010, 3.5]

3.2
culture medium
nutrients presented in a form and phase (liquid or solidified) which support microbiological growth

3.3
dilution level
D
denominator of the dilution coefficient (using the numerator 1) of a mixture of water or waste water with dilution water as integral number

Note 1 to entry: For undiluted water or waste water, this coefficient per definition is 1→1. The corresponding and smallest possible value of *D* is 1. In this document, the arrow indicates the transition from initial total volume to final total volume.

[SOURCE: ISO 6107-6:2004, 28]

3.4
dilution water
water added to the test sample to prepare a series of defined dilutions

[SOURCE: ISO 20079:2005, 3.7]

3.5
50 % effect concentration
EC_{50}
concentration of a compound which causes 50 % of an effect

Note 1 to entry: In the sense of this document the EC_{50} is the concentration of a compound which induces 50 % of the maximal reporter gene activity which can be achieved by this compound.

3.6
field blank
container prepared in the laboratory, using reagent water or other blank matrix, and sent with the sampling personnel for exposure to the sampling environment to verify possible contamination during sampling

[SOURCE: ISO 11074:2015, 4.5.3]

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3.7
growth rate
proportional rate of increase in cell density

[SOURCE: ISO 10253:2006, 3.2]

3.8
induction rate
quotient of the mean value of wells with enhanced reporter gene activity measured on the plates treated with a dose of the test sample, and the mean value of the corresponding wells treated with the negative control using the same strain under identical conditions

Note 1 to entry: Instead of the negative control, the estimated parameter A of the four-parameter model, which describes the dose response relationship between reference compound and the induction rate, can be used.

[SOURCE: ISO 6107-6:2004, 43, modified — "wells with enhanced reporter gene activity measured" replaces "mutant colonies"; "corresponding wells" replaces "corresponding plates"; “quotient” replaces “difference”.]

3.9
inoculum
fraction of a culture of microorganisms used to start a new culture, or an exponentially growing preculture, in fresh medium

[SOURCE: ISO 6107-6:2004, 44]

3.10
lowest ineffective dilution value
LID
lowest dilution within a test batch which does not show any effect, i.e. no statistically significant increase in the reporter gene activity compared with the negative control

[SOURCE: ISO 11350:2012, 3.4, modified — “increase in the reporter gene activity” replaces “increase in the number of revertant wells”.]

3.11
negative control
dilution water without test sample

[SOURCE: ISO 6107-6:2004, 51]

3.12
reference compound
compound with one or more property values that are sufficiently reproducible and well established to enable the calibration of the measurement method

[SOURCE: ISO 7405:2008, 3.6, modified — “compound” replaces “material”; “the calibration of the measurement method” replaces “use of the material or substance for the calibration of an apparatus, the assessment of a measurement method or for the assignment of values to materials”.]

3.13
reporter gene activity
quantitative activity of a gene attached to the promoter sequence of another gene

3.14
test sample
undiluted, diluted or otherwise prepared portion of a sample to be tested, after completion of all preparation steps such as centrifugation, filtration, homogenization, pH adjustment and determination of ionic strength

[SOURCE: ISO 6107-6:2004, 92]

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