
**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*)*

ISO 19040-2:2018

<https://standards.iteh.ai/catalog/standards/iso/9ef13823-4086-4a56-86cf-334c1492bbb7/iso-19040-2-2018>



iTeh Standards
(<https://standards.iteh.ai>)
Document Preview

ISO 19040-2:2018

<https://standards.iteh.ai/catalog/standards/iso/9ef13823-4086-4a56-86cf-334c1492bbb7/iso-19040-2-2018>



COPYRIGHT PROTECTED DOCUMENT

© ISO 2018

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
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword	v
1 Scope	1
2 Normative references	2
3 Terms and definitions	2
4 Principle	4
5 Interferences	4
6 Apparatus and materials	4
7 Reagents, media and test strains	5
8 Sampling and samples	9
8.1 General	9
8.2 Bottles and material for sampling	9
8.3 Bottles and material pre-cleaning	10
8.4 Sampling procedure	10
8.5 Transport of samples	10
8.6 Pretreatment of samples	10
8.7 Storage of samples	11
9 Procedure	11
9.1 Test set up	11
9.1.1 Preparation of the reference dilution series	11
9.1.2 Reactivation of the yeast	11
9.1.3 Negative control	12
9.1.4 Blank replicate	12
9.1.5 Sample dilution	12
9.1.6 Field blank	13
9.1.7 Plate set up	13
9.1.8 Inoculation of the test plate	13
9.2 Measurement	14
9.2.1 Measurement of the reporter gene activity	14
9.2.2 Measurement of the cell density	15
9.3 Calculations	15
9.3.1 Background correction	15
9.3.2 Calculation of the relative growth	16
9.3.3 Calculations for assessment of sample blanks	17
9.3.4 Calculation of the reporter gene induction	19
10 Validity criteria	22
11 Assessment criteria	23
12 Test report	23
13 Verification	23
Annex A (informative) Plate set up	25
Annex B (informative) Lyophilization of <i>Arxula adeninivorans</i> cells	26
Annex C (informative) Scheme of test principle	28
Annex D (informative) Test set up for chemicals and extracts	29
Annex E (informative) Preparation of dilution series	30
Annex F (informative) Performance data	31
Annex G (informative) Statistical assessment	41

Annex H (informative) Calculation of estradiol equivalents	42
Annex I (informative) Alternative test design for EEQ determination	45
Annex J (informative) Measurement of the lowest ineffective dilution (LID) of a waste water — A simplified evaluation for testing of waste water	46
Annex K (informative) Example for statistical evaluation	48
Bibliography	55

iTeh Standards
(<https://standards.iteh.ai>)
Document Preview

ISO 19040-2:2018

<https://standards.iteh.ai/catalog/standards/iso/9ef13823-4086-4a56-86cf-334c1492bbb7/iso-19040-2-2018>

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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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.

[ISO 19040-2:2018](https://standards.iteh.ai/ISO/19040-2:2018)

<https://standards.iteh.ai/catalog/standards/iso/9ef13823-4086-4a56-86cf-334c1492bbb7/iso-19040-2-2018>

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 17 β -estradiol equivalents (EEQ). The upper threshold of the dynamic range for this test is between 25 ng/l and 40 ng/l 17 β -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.