BS EN ISO 19040-1:2022
Incorporating corrigendum October 2022

BSI Standards Publication

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

Part 1: Yeast estrogen screen (Saccharomyces cerevisiae)

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee EH/3/5, Biological Methods.

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Published by BSI Standards Limited 2022

ISBN 978 0 539 21522 9

ICS 13.060.70

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 August 2018.

Amendments/corrigenda issued since publication

Date	Text affected
31 October 2022	This corrigendum renumbers BS ISO 19040-1:2018 as BS EN ISO 19040-1:2022

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 19040-1

September 2022

ICS 13.060.70

English Version

Water quality - Determination of the estrogenic potential of water and waste water - Part 1: Yeast estrogen screen (Saccharomyces cerevisiae) (ISO 19040-1:2018)

Qualité de l'eau - Détermination du potentiel oestrogénique de l'eau et des eaux résiduaires - Partie 1: Essai d'oestrogénicité sur levures (Saccharomyces cerevisiae) (ISO 19040-1:2018)

Wasserbeschaffenheit - Bestimmung des estrogenen Potentials von Wasser und Abwasser - Teil 1: Hefe-Estrogenscreening (Saccharomyces cerevisiae) (ISO 19040-1:2018)

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European foreword

The text of ISO 19040-1:2018 has been prepared by Technical Committee ISO/TC 147 "Water quality" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 19040-1:2022 by Technical Committee CEN/TC 230 "Water analysis" the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by March 2023, and conflicting national standards shall be withdrawn at the latest by March 2023.

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Foreword

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

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

Part 1:
Yeast estrogen screen (*Saccharomyces cerevisiae*)

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 genetically modified yeast strains *Saccharomyces cerevisiae*. This reporter gene assay is based on the activation of the human estrogen receptor alpha.

This method is applicable to:

— fresh water;

— waste 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 8 ng/l and 15 ng/l 17β-estradiol equivalents (EEQ) based on the results of the international interlaboratory trial (see Annex F). The upper threshold of the dynamic range for this test is between 120 ng/l and 160 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.

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*

ISO 7027, *Water quality — Determination of turbidity*

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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 http://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

[SOURCE: ISO 6107-6:2004, 24]

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
induction rate
quotient of the mean signal measured after exposure to a dose of the test sample or with a positive control, and the mean signal measured for the negative control using the same experimental conditions

[SOURCE: ISO 6107-6:2004, 43, modified — “corrected absorbance” replaces “mutant colonies”; “wells” replaces “corresponding plates”, “quotient” replaces “difference”.]

3.8
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]

3.9
limit of quantification
LOQ
lowest value of a determinant that can be determined with an acceptable level of accuracy and precision

[SOURCE: ISO 15839:2003, 3.18]

3.10
lowest ineffective dilution
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
overnight culture
culture started late in the afternoon and incubated overnight to be ready during the following morning for purposes such as the inoculation of a preculture

Note 1 to entry: The procedure for the overnight culture is described in 9.2.

[SOURCE: ISO 6107-6:2004, 54, modified — deleted: "usually about 16 h".]

3.13
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”.]

3.14
reporter gene activity
quantitative activity of a gene attached to the promoter sequence of another gene

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3.15
stock culture
culture of a strain of organisms maintained under conditions to preserve original features such as nucleotide sequences

[SOURCE: ISO 6107-6:2004, 87]

3.16
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]

4 Principle

The Yeast Estrogen Screen (YES) is a reporter gene assay which can be used for the measurement of the activation of the human estrogen receptor alpha (hERα) in the presence of a sample containing compounds which activate the estrogen receptor (ER).

By this means the assay detects the estrogenic activity of the whole sample in its actual state as an integral measure including possible additive, synergistic and antagonistic mixture-effects on the whole process of the reporter gene expression.

The basic concept of such assays is explained in References [10] and [11]. The hERα is heterologously expressed in the yeast cell under control of a copper dependent promoter. The estrogen receptor belongs to the family of nuclear hormone receptors. If agonists of the estrogen receptor enter the yeast cell, they bind to the estrogen receptor protein and thus induce its conformational change. As a consequence two receptor proteins form a receptor dimer which translocates to the nucleus. This activation of the estrogen receptor is measured by the induction of the reporter gene *lacZ* which encodes the enzyme β-galactosidase. The *lacZ* is fused to a promoter containing estrogen responsive elements (ERE) and is thus controlled by the activity of the estrogen receptor. The ER-dimer binds to the promoter and by this activates the expression of the β-galactosidase. Finally, the activity of the β-galactosidase as a measure for the estrogenic potential of the sample is determined using an appropriate substrate which is cleaved to a coloured reaction product. The reaction product can be measured photometrically. See Annex C for a scheme of the test principle.

5 Interferences

Coloured or turbid samples might interfere with the photometric detection of cell density and/or the detection of the reaction product of the reporter enzyme β-Galactosidase (see Clause 10 for further information).

Toxic effects of the test sample may lead to a reduction of viable cells and to a reduction of the measurable signal. Consequently, estrogenic effects of a sample may be masked by acute toxic effects leading to false negative test results (see Clause 10 for further information).

High salinity can cause toxic effects due to the resulting osmotic pressure. The conductivity of the sample is a measure for its salinity. The yeast strain constructed by McDonnell et al. (Reference [10]) tolerates a conductivity of the sample up to 34 000 µS/cm.

Bacterial growth in the test wells is assessed by the blank replicate (3.1). See Clause 10 for further information.

If filtered samples are tested in order to remove bacteria from the sample, solid particles are separated from the sample also. Thus, substances with estrogenic activity which are adsorbed on particles might not be detected.

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Due to the high sensitivity of this test avoid any contamination of buffer, media and all reagents used with compounds exhibiting estrogenic activity to avoid false positive test results.

6 Apparatus and materials

For suitable sampling devices see Clause 8. Usual laboratory apparatus and glassware is required. In particular, the following material is needed:

6.1 Incubator shaker, temperature- and time-controlled, 30 °C ± 1 °C and 37 °C ± 1 °C.

6.2 pH meter.

6.3 Steam sterilizer.

6.4 Dry sterilizer.

6.5 Centrifuge, with a rotor for 15 ml and 50 ml tubes up to 2 500 *g* and with a rotor for 96-well plates up to 2 500 *g*.

6.6 Rotary mixer.

6.7 Freezer, at least ≤−18 °C and ≤−70 °C.

6.8 Sterile filter, cellulose acetate, 0,2 µm pore size.

6.9 Inoculation loops.

6.10 Multi-channel multistepper pipette (repeater pipette).

6.11 Multi-channel pipettes, 5 µl to 50 µl and 50 µl to 300 µl.

6.12 Spectrophotometer.

6.13 Transparent sterile polystyrene 96-well plates, for suspension cultures with flat bottom and lid.

6.14 Microplate photometer for 96-well plates, for absorbance measurement at 540 nm ± 20 nm or 580 nm ± 20 nm and at 600 nm ± 20 nm.

6.15 Clean bench.

6.16 Petri dishes, diameter approximately 94 mm, height approximately 16 mm.

6.17 Cryogenic vials, sterile, 1 ml, 10 ml.

6.18 Disposable nitrile gloves.

6.19 Air-permeable sealing membranes for 96-well plates.

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7 Reagents, media and test strain

7.1 Reagents

As far as possible, use "reagent grade" chemicals.

7.1.1 Yeast nitrogen base without amino acids[1].

7.1.2 α-D-Glucose, anhydrous, $C_6H_{12}O_6$, molecular weight 180,15 g/mol, CAS: 50-99-7.

7.1.3 Adenine, $C_5H_5N_5$, molecular weight 135,13 g/mol, CAS: 73-24-5.

7.1.4 L-arginine, $C_6H_{14}N_4O_2$, molecular weight 174,20 g/mol, CAS: 74-79-3.

7.1.5 L-aspartic acid, $C_4H_7NO_4$, molecular weight 133,10 g/mol, CAS: 56-84-8.

7.1.6 L-glutamic acid monosodium salt hydrate, $C_5H_8NNaO_4{\cdot}H_2O$, molecular weight (anhydrous) 169,11 g/mol, CAS: 142-47-2 (anhydrous basis).

7.1.7 L-histidine-HCl, $C_6H_9N_3O_2{\cdot}HCl{\cdot}H_2O$, molecular weight 209,6 g/mol, CAS: 5934-29-2.

7.1.8 L-isoleucine, $C_6H_{13}NO_2$, molecular weight 131,17 g/mol, CAS: 73-32-5.

7.1.9 L-leucine, $C_6H_{13}NO_2$, molecular weight 131,17 g/mol, CAS: 61-90-5.

7.1.10 L-lysine-HCl, $C_6H_{14}N_2O_2{\cdot}HCl$, molecular weight 182,65 g/mol, CAS: 657-27-2.

7.1.11 L-methionine, $C_5H_{11}NO_2S$, molecular weight 149,21 g/mol, CAS: 63-68-3.

7.1.12 L-phenylalanine, $C_9H_{11}NO_2$, molecular weight 165,19 g/mol, CAS: 63-91-2.

7.1.13 L-serine, $C_3H_7NO_3$, molecular weight 105,09 g/mol, CAS: 56-45-1.

7.1.14 L-threonine, $C_4H_9NO_3$, molecular weight 119,12 g/mol, CAS: 72-19-5.

7.1.15 L-tyrosine, $C_9H_{11}NO_3$, molecular weight 181,19 g/mol, CAS: 60-18-4.

7.1.16 L-valine, $C_5H_{11}NO_2$, molecular weight 117,15 g/mol, CAS: 72-18-4.

7.1.17 Copper(II) sulfate pentahydrate, $CuSO_4{\cdot}5H_2O$, molecular weight 249,69 g/mol, CAS: 7758-99-8.

7.1.18 Ampicillin sodium salt, $C_{16}H_{18}N_3NaO_4S$, molecular weight 371,39 g/mol, CAS: 69-52-3.

7.1.19 Streptomycin sulfate salt, $C_{21}H_{39}N_7O_{12}{\cdot}1{,}5H_2SO_4$, molecular weight 728,69 g/mol, CAS: 3810-74-0.

7.1.20 Agar for microbiology, $(C_{12}H_{18}O_9)n$, CAS: 9002-18-0.

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1) Yeast nitrogen base without amino acids contains a nitrogen source such as ammonium sulfate, vitamins and trace elements which are required for growth of yeast cells. Yeast nitrogen base without amino acids is used for the selection of yeast strains depending on requirements for carbon sources and amino acids.

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7.1.21 Hydrochloric acid solution, 1 M (HCl), molecular weight 36,46 g/mol, CAS: 7647-01-0.

7.1.22 Sodium hydroxide, NaOH, molecular weight 40,00 g/mol, CAS: 1310-73-2.

7.1.23 Ethanol, ≥99,8 %, CH_3CH_2OH, molecular weight 46,07 g/mol, CAS: 64-17-5.

7.1.24 Glycerol for molecular biology, ≥99 %, $HOCH_2CH(OH)CH_2OH$, molecular weight 92,09 g/mol, CAS: 56-81-5.

7.1.25 17*β*-Estradiol, ≥98 %, $C_{18}H_{24}O_2$, molecular weight 272,38 g/mol, CAS: 50-28-2.

7.1.26 Disodium hydrogen phosphate dihydrate, $Na_2HPO_4{\cdot}2H_2O$, molecular weight 177,99 g/mol, CAS: 10028-24-7.

7.1.27 Sodiumdihydrogen phosphate monohydrate, $NaH_2PO_4{\cdot}H_2O$, molecular weight 137,99 g/mol, CAS: 10049-21-5.

7.1.28 Potassium chloride, KCl, molecular weight 74,55 g/mol, CAS: 7447-40-7.

7.1.29 Magnesium sulfate heptahydrate, $MgSO_4{\cdot}7H_2O$, molecular weight 246,47 g/mol, CAS: 10034-99-8.

7.1.30 Chlorophenolred-β-D-galactopyranoside (CPRG), $C_{25}H_{22}Cl_2O_{10}S$, molecular weight 585,41 g/mol, CAS: 99792-79-7.

7.1.31 Lyticase from *Arthrobacter luteus* lyophilized powder, ≥2 000 units/mg protein, CAS: 37340-57-1.

7.1.32 DL-Dithiothreitol, $HSCH_2CH(OH)CH(OH)CH_2SH$, molecular weight 154,25 g/mol, CAS: 3483-12-3.

7.1.33 Sodium dodecyl sulfate, $CH_3(CH_2)_{11}OSO_3Na$, molecular weight 288,38 g/mol, CAS: 151-21-3.

7.1.34 Aceton (puriss p.a.), CH_3COCH_3, molecular weight 58,08 g/mol, CAS: 67-64-1.

7.2 Water, grade 3, as defined in ISO 3696; water with a conductivity up to 5 µS/cm is acceptable.

If sterile water is needed, autoclave or sterilize by filtration (cellulose acetate, 0,2 µm). Water as specified here is also used for the stepwise dilution of the test sample.

7.3 Test strain.

The generation of the test strain is described in References [10] and [11]. It is derived from the strain *Saccharomyces cerevisiae* BJ3505 (protease deficient, MAT*α*, PEP4::HIS3, prb-1-delta1.6R, HIS3-delta200, lys2-801, trp1-delta101, ura3-52gal2can1). This strain harbours two plasmids. The construction of these plasmids is described in Reference [10]. The plasmid YEPE10 contains the *CUP1::hER* fusion which encodes the human estrogen receptor *α* cloned from the MCF-7 human cell lineage under the control of the metallothionein promoter *CUP1*. This plasmid is selected via the tryptophane auxothropy of the parent strain. The second plasmid is the reporter plasmid YRPEG3 which contains the fusion gene *2ERE-CyC1::lacZ*. This fusion gene expresses the *β*-galactosidase (encoded by *lacZ*) under the control of the iso1cytochrom c promoter from *S. cerevisiae* which is fused to two copies of the vitellogenin A2-gene from *Xenopus laevis*. This plasmid is selected via the uracil auxothropy of the parent strain.

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7.4 Media.

If required autoclave for 20 min at 121 °C ± 2 °C. Cover the vessels loosely (e.g. with aluminium foil). Never seal air-tight.

7.4.1 10x SD-Medium.

Dissolve 67,0 g yeast nitrogen base without amino acids (7.1.1) and 200 g glucose (7.1.2) in water (7.2) and adjust the final volume with water (7.2) to 1 l. Sterilize the solution by filtration (cellulose acetate, 0,2 µm). Do not autoclave the solution because it contains vitamins such as Biotin. Store the solution in 50 ml aliquots in the dark at ≤ −18 °C no longer than 12 months.

7.4.2 10x McD-DO-Medium (McDonnell).

Dissolve

— 175 mg L-lysine-HCl (7.1.10);

— 120 mg L-histidine-HCl (7.1.7);

in water (7.2) and adjust the final volume with water (7.2) to 500 ml.

Sterilize by filtration (cellulose acetate, 0,2 µm) and store aliquots of 40 ml in the dark at ≤−18 °C no longer than six months.

7.4.3 Glucose solution.

Dissolve

— 144 g α-D-Glucose (7.1.2)

in water (7.2) and adjust the final volume with water (7.2) to 500 ml.

Sterilize by filtration (cellulose acetate, 0,2 µm) or autoclave and store the solution in the dark at 2 °C to 8 °C no longer than 12 months.

7.4.4 $CuSO_4$ solution, 10 mmol/l.

Dissolve 250 mg $CuSO_4 \cdot 5H_2O$ (7.1.17) in water (7.2) and adjust the final volume with water (7.2) to 100 ml.

Autoclave the solution. The solution may be used up to 12 months at room temperature.

7.4.5 Ampicillin stock solution.

Dissolve 1 g ampicillin sodium salt (7.1.18) in water (7.2) and adjust the final volume with water (7.2) to 10 ml.

Sterilize the solution by filtration (cellulose acetate, 0,2 µm) and store 1 ml aliquots in the dark at ≤−18 °C no longer than 12 months.

7.4.6 Streptomycin stock solution.

Dissolve 1 g streptomycin sulfate salt (7.1.19) in water (7.2) and adjust the final volume with water (7.2) to 10 ml.

Sterilize the solution by filtration (cellulose acetate, 0,2 µm) and store 1 ml aliquots in the dark at ≤−18 °C no longer than 12 months.

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7.4.7 Growth medium (McDonnell).

Add

— 10 ml 10x SD-Medium (7.4.1);

— 10 ml 10x McD-DO-Medium (McDonnell, 7.4.2);

to 80 ml sterile water (7.2) under sterile conditions. Split the medium in 20 ml aliquots. Store the medium in the dark at 2 °C to 8 °C no longer than one week or in the dark at ≤–18 °C no longer than six months.

7.4.8 Exposure medium (McDonnell).

Mix

— 4,2 ml 10x SD-Medium (7.4.1);

— 4,2 ml 10x McD-DO-Medium DO-Medium (7.4.2);

— 1,6 ml Glucose solution (7.4.3);

— 99 µl $CuSO_4$-solution (7.4.4);

— 67 µl Ampicillin stock solution (7.4.5);

— 67 µl Streptomycin stock solution (7.4.6).

Prepare the exposure medium immediately before use. A volume of 5 ml per 96-well plate is required.

7.4.9 Aqueous solution of ethanol, volume fraction 0,3 %.

Add sterile water (7.2) to 300 µl ethanol (7.1.23) to a final volume of 100 ml.

7.4.10 Aqueous solution of glycerol, volume fraction 30 %.

Add water (7.2) to 3 ml glycerol (7.1.24) to a final volume of 10 ml. Sterilize the solution by filtration (Cellulose acetate, 0,2 µm) or autoclave.

7.4.11 17*β*-estradiol (E2) stock solution.

Dissolve 50 mg 17*β*-estradiol (E2) (7.1.25) in ethanol (7.1.23) and adjust the final volume with ethanol (7.1.23) to 10 ml. Store the stock solution in aliquots of 1 ml in the dark at ≤ –18 °C no longer than 12 months.

7.4.12 lacZ buffer.

Dissolve in 950 ml of water (7.2):

— 10,67 g $Na_2HPO_4 \cdot 2H_2O$ (7.1.26);

— 0,75 g KCl (7.1.28);

— 0,25 g $MgSO_4 \cdot 7H_2O$ (7.1.29).

Before the adjustment of the pH the solution might be turbid. Adjust the pH to pH = 7,0 ± 0,2 by the addition of $NaH_2PO_4 \cdot H_2O$ (7.1.27) (approximately 5,5 g).

Add and dissolve

— 1 g sodium dodecyl sulfate (7.1.33).

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Adjust the final volume to 1 000 ml with water (7.2). Store the solution at room temperature no longer than six months.

7.4.13 lacZ reaction mixture

Add and dissolve per ml lacZ buffer:

— 0,4 mg chlorophenolred-β-D-galactopyranoside (CPRG) (7.1.30);

— 250 U Lyticase (7.1.31);

— 0,154 mg DL-Dithiothreitol (7.1.32).

For one 96-well plate 6 ml lacZ-reaction mixture are required. Prepare the lacZ-reaction mixture immediately before use.

Different batches of the lyticase may have a different specific lyticase-activity. Calculate the amount of lyticase needed for the reaction mixture according to:

$$m_{\text{lyticase}} = \frac{250\ \text{U}}{A_s} \tag{1}$$

where

m_{lyticase} is the mass (mg) of lyticase with a specific activity of A_s which is required for 1 ml lacZ-buffer;

A_s is the specific activity of the lyticase in U/mg.

8 Sampling and samples

8.1 General

This clause describes specific requirements for the sampling with respect to the determination of estrogenic activity in water samples. For general information about sampling consider ISO 5667-16.

8.2 Bottles and material for sampling

Use clean glass bottles (borosilicate glass) with polytetrafluoroethylene (PTFE)-lined caps. To avoid photo-degradation of compounds of interest, use amber glass bottles. If transparent glass bottles are used, wrap the bottles in aluminium foil or store them in a dark container.

Alternatively, bottles made from aluminium or stainless steel (both uncoated) may be used. Assess that a material different from borosilicate glass does not affect results.

8.3 Bottles and material pre-cleaning

After the routine cleaning procedure, additionally clean the bottles and the caps as follows: rinse the clean bottles and the caps three times with a minimum amount of acetone (7.1.34). Let the residual acetone evaporate (e.g. drying oven). Close the bottles immediately after drying. Rinse all glassware, spatulas etc. getting in contact with the sample three times with a minimum amount of acetone (7.1.34). Let the residual acetone evaporate.

8.4 Sampling procedure

Use disposable nitrile-gloves during sampling. Do not use any hand-cream prior to sampling and avoid skin contact with the sample. Use material from glass, PTFE, aluminium or stainless steel only.

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