XENOMETRIX

Swiss Commitment for Bioassays

XenoScreen XL YES/YAS

Accelerated high-sensitivity microplate assay for the detection of compounds with estrogenic and androgenic agonistic and antagonistic activities

using *Saccharomyces cerevisiae* strains with human estrogen (hERα) and androgen (hAR) receptors

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Short protocol

For 4 × 96 data points

Art. No. N04-233-Y

Upon receipt of your XenoScreen XL YES/YAS Assay kit, **make sure that all reagents are stored appropriately (see pg. 4 for storage instructions)**. If components are damaged or if any problems occur, please contact Xenometrix by phone: ++41-61-482-14-34; fax: ++41-61-482-20-72, or e-mail: info@xenometrix.ch

For Research use only **Version 3.06** **May 2018**

Changelog

Date	New version	Changes
16.12.2013	2.22	• Changelog added • Final incubation 30 or 60 minutes: additional information (p. 12)
10.2.2014	2.23	• Consistent use of the term 'lacZ lysis buffer' throughout the document. • Relaxed OD_{690} requirement for acceptable starting cell density from 0.3 to 0.2 (page 9) • Additional handling tips for the lacZ reaction buffer and air bubbles (page 12). • Optimized recommendations for the storage and handling of yeast cells in Appendix A
15.7.2014	2.24	• Recommendation to extend exposure from 18 hrs if growth of cells was insufficient (page 12) • New Xenometrix logo
2.9.2014	2.25	• New more realistic numbers for calculation example on page 11 • Pre-warmed media recommended for dilutions of growing cells (page 6+10).
21.10.2014	2.26	• More details on regulations for the use of GMO's (Safety Precautions, page 2)
18.11.2014	3.00	• Shelf life of complete growth medium added (page 6) • Corrected plate layout • Expandend data evaluation for Excel Calculation Sheet versions 3 and up • Added additional option to send in raw data for evaluation
17.3.2015	3.01	• 4 T25 flask instead of 2
8.10.2015	3.02	• New order number for Growth Medium
30.5.2016	3.03	• Suggestion to prepare own YES **and** YAS stocks added
20.6.2016	3.04	• Added Art. Nr. of kit version without strains
5.12.2016	3.05	• Removed strains from "Kit contents"; yeast cells have to be purchased separately
23.5.2018	3.06	• Minor wording optimization • New Assay Procedure Scheme.

XenoScreen XL YES/YAS Assay Kit
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Number of Data Points

This test provides a total of 4× 96 data points (2× 96 YES and 2× 96 YAS).
We recommend to run test samples in duplicates in 8 concentrations in order to obtain dose-response curves for the calculation of estrogen and androgen agonist and antagonist activities.

The assay description in this manual is based on such a complete analysis which allows to test 4 samples for estrogenic, anti-estrogenic, androgenic and anti-androgenic activities with all necessary controls, the calculation of EEQ and AEQ and the determination of the Limit of Detection LoD and the Limit of Quantification LoQ.

The free Excel calculation workbook available from Xenometrix is based on the plate layout described in this manual.

Principle of the Test

The common Baker's or Brewer's yeast (*Saccharomyces cerevisiae*) was genetically modified to identify compounds that can interact with the human estrogen and androgen receptors hERα and hAR. For this purpose the DNA sequences of hERα or hAR were stably integrated into the main chromosome of yeast cells. Additionally, the cells also contain an expression plasmid carrying the reporter gene lacZ encoding the enzyme β-galactosidase and estrogen (YES) or androgen (YAS) responsive elements (Routledge, E.J. and Sumpter, J.P. 1996. *Environ. Toxicol. Chem*. **13**:241–8; Sohoni, P. and Sumpter, J.P. 1998. *Endocrinol*. **158**:327–39)

Upon binding of a ligand, the hERα and hAR interact with the corresponding response elements on the expression plasmid and modulate the transcription of the lacZ reporter gene. The β-galactosidase is secreted into the medium and converts the yellow substrate chlorophenol red-β-D-galactopyranoside (CPRG) into red product which can be quantified colorimetrically at 570 nm. The measured OD_{570} correlates directly with the amount of secreted β-galactosidase and thus with the activity of the test substance which binds to the corresponding receptor.

The XenoScreen XL YES/YAS assay system can identify both activating (agonistic) and inhibitory (antagonistic) activities of test compounds. For the determination of antagonist activities, the samples are incubated in the presence of a fixed concentration of a reference agonist (17-β estradiol for YES and 5α-dihydrotestosterone for YAS). Inhibition of the response relative to this fixed agonist concentration is a sign of antagonist activity.

The assay can be used for either water samples or for samples dissolved in a solvent like DMSO. Samples dissolved in a solvent have to be diluted 100x in the assay in order to have acceptable levels of solvent.

The XenoScreen XL YES/YAS uses lyticase and a detergent (=LYES and LYAS) to facilitate the secretion of the intracellularly synthesized β-galactosidase (Schultis T. and Metzger J.W., 2004. *Chemosphere*. **57**:1649–55). This allows to reduce the incubation time from 48 hrs in the standard YES/YAS assay to 18 hours. In addition the accelerated protocol leads also to enhanced sensitivities for estrogenic and androgenic compounds.

Assay Description

Growing yeast cells stably transformed with either hERα (YES) or hAR (YAS) and a β-galactosidase reporter system are exposed to serial dilutions of test compound, positive control chemicals (17-β estradiol for YES and 5α-dihydrotestosterone for YAS) and a combination of a fixed concentration of positive control chemical and serial dilutions of the test compound. The cells are incubated for 18 hrs at 31°C. The induced cells are lysed in the presence of the yellow substrate CPRG which turns purple in the presence of β-galactosidase. Yeast cell growth is assessed prior to addition of the lysis buffer at an OD_{690}. The color development is measured at 570 nm and is corrected for diffraction by cells and debris by a simultaneous measurement of OD_{690}. The results are evaluated for estrogenic and androgenic agonistic and antagonistic activities, as well as for yeast growth inhibition or cytotoxicity.

Safety Precautions

- The YES and YAS yeast cells are genetically modified organisms (GMO). Please consult with your institutional and regulatory authorities for the requirements for handling, storage and disposal of such organisms in accordance with directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms (replaces Council Directive 90/219/EEC of 23 April 1990).
- The control chemicals provided in this kit are hormonally active substances. Please consult the Material and Safety Data Sheets (MSDS) for information on handling, disposal and personal protection.
- Not for use in humans and animals. For research purposes only.
- Do not drink, eat, smoke, or apply cosmetics in designated work areas. Wear laboratory coats and gloves when handling specimens and kit reagents. Wash hands thoroughly afterwards. Do not pipette by mouth.

Warnings

» Please observe all highlighted warnings and hints in the text! «

- **Due to the high sensitivity of the XenoScreen XL YES/YAS assay all containers and pipettes coming into contact with the cells or reagents must be absolutely clean and devoid of any residual chemicals such as detergents.**
- **When reusable items are used they should be thoroughly rinsed with distilled water and ethanol (without any additives). We highly recommend to wear gloves also for the handling of glassware and plasticware.**
- **All solvents should be of the highest available purity grade without any additives.**
- **Read the whole Instructions for Use before starting the assay!**

Kit Components

Each XenoScreen XL YES/YAS assay kit contains media and reagents for the analysis of 4 test compounds for agonistic and antagonistic estrogenic (YES) and androgenic (YAS) endocrine activity.

Use your own aliquots of YES and YAS yeast cells or order them separatley: XenoScreen YES Strain, 1 vial, Art. No. N05-230-E; XenoScreen YAS Strain, 1 vial, Art. No. N05-230-A

The compounds are tested in 8 dilutions. Each assay has its own positive and negative controls.

Alternative plate layouts, dilution schemes or replicate numbers are possible, but are not described in this manual and are not supported by the Excel calculation sheet provided by Xenometrix.

Kit contents:

- Basal medium
- Vitamin solution
- L-aspartic acid solution
- L-threonine solution
- Cu-sulfate solution (500 µl)
- 10× DO medium
- 10× SD medium
- Streptomycin/Ampicillin solution
- DMSO
- Substrate solution CPRG
- 1 vial with 17β-estradiol positive control (“E2”, red label; YES)
- 1 vial with 5α-dihydrotestosterone positive control (“DHT”, blue label; YAS)
- 1 vial with 4-hydroxytamoxifen control antagonist (“HT”, yellow label; YES)
- 1 vial with flutamide control antagonist (“FL”, green label; YAS)
- 1 vial with lyticase
- 1 vial with lacZ lysis buffer
- 1 vial with 2-mercaptoethanol
- 9 96-well plates (2×4 for the assay, 1 for measurement of cell densities)
- 4 gas-permeable plate sealers
- 4 T25 culture flasks with gas-permeable filter caps

To be ordered separately:

- XenoScreen YES Strain, 1 vial, Art. No. N05-230-E
- XenoScreen YAS Strain, 1 vial, Art. No. N05-230-A

Storage Conditions

Each Xenometrix XenoScreen XL YES/YAS kit is shipped at ambient temperature. Please contact Xenometrix if you received the kit later than 10 days after the shipment date indicated on the delivery note (phone: ++41-61-482-14-34; fax: ++41-61-482-20-72, or e-mail: info@xenometrix.ch).

The shipment contains the following components which should be stored **immediately upon arrival** as follows:

–20°C:

- Positive and antagonistic controls before reconstitution (4 vials, E2, DHT, 4-HT, FL)
- Lyticase
- Streptomycin/Ampicillin
- *If ordered separately: Yeast cells on filter discs when stored for more than 1 month*

2 – 8°C

- CPRG substrate solution
- Basal medium
- Vitamin solution
- L-threonine solution
- 2-mercaptoethanol
- 10× DO medium
- 10× SD medium
- Positive controls and antagonist controls after reconstitution
- *If ordered separately: Yeast cells on filter discs when used within 1 month*

20 – 25°C (room temperature, liquids protected from light):

- L-aspartic acid solution
- Cu(II)-sulfate solution
- DMSO
- lacZ lysis buffer
- 96-well plates
- Culture flasks
- Plate sealers

Required Equipment and Consumables NOT Included with the Kit

- XenoScreen YES Strain, 1 vial, Art. No. N05-230-E
- XenoScreen YAS Strain, 1 vial, Art. No. N05-230-A
- Incubator (31°C) with rotating platform (orbital movement)
- High-humidity container (e.g. plastic box with a tight lid, and wet paper tissue)
- Microplate reader capable to read at 570 and 690 nm
- Adjustable micropipettes and sterile tips
  (needed volumes: 2, 20–200, 100–1000 µl)
- Adjustable 8-channel pipettes (needed volumes: 20–200 µl)
- Serological pipettes (sterile)
- Pipetting reservoirs (sterile)
- Gloves
- Freezing tubes for the establishment of your own yeast stocks
- Glycerol for freezing cells

Recommended:

- Inverted microscope to inspect yeast cultures

XenoScreen XL – Assay Procedure

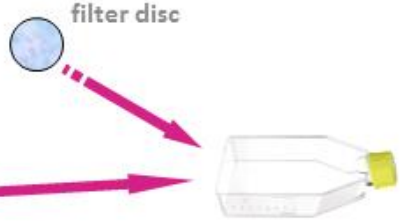
Day -4 – Day -2:

Growth Medium Preparation

Supplements

Basal medium

Yeast cells on filter disc



2 – 4 days
31°C
100 rpm

Yeast culture growth

Day 1:

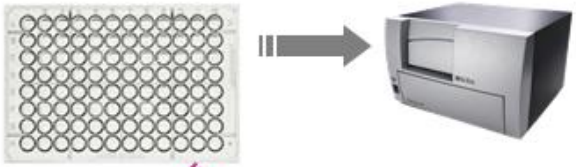
1. Dilution Preparation
directly in assay plate (samples, controls)
2. Addition of yeast cells.

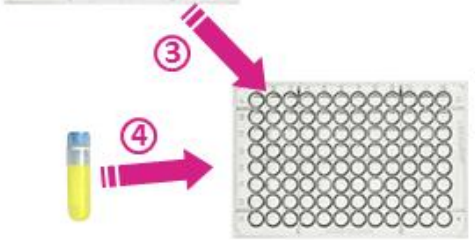
18 hours
31°C
100 rpm

Incubation in a sealed, humid box

Day 2:

OD_{690} Reading





30 – 60 min

OD_{570} Reading
OD_{690} Reading

3. Transfer to fresh plates
4. Addition of substrate in Lysis Medium

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Assay Procedure