



XenoScreen XL YES

Accelerated high-sensitivity microplate assay for the detection of compounds with estrogenic activity

using *Saccharomyces cerevisiae* strains with human estrogen (hER α) receptor

Short protocol

**For 96 data points
Art. No. N32-233-Y**

Upon receipt of your XenoScreen XL YES 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

Change log

Date	New version	Changes
22.09.2014	1.01	<ul style="list-style-type: none">• First version, derived from XenoScreen XL YES/YAS version 3.0
15.12.2014	1.02	<ul style="list-style-type: none">• Textual clarifications, improved formatting
05.01.2015	1.03	<ul style="list-style-type: none">• Added additional option to send in raw data for evaluation
17.03.2015	1.04	<ul style="list-style-type: none">• 4 T25 flask instead of 2
08.10.2015	1.05	<ul style="list-style-type: none">• New order number for Growth Medium
12.10.2016	1.06	<ul style="list-style-type: none">• Corrected number of T25 flasks in kit (2 instead of 4)
06.12.2016	1.07	<ul style="list-style-type: none">• Removed strains from “Kit contents”; yeast cells have to be purchased separately• New order number
23.05.2018	2.0	<ul style="list-style-type: none">• Minor wording optimization• New Assay Procedure graphics.

XenoScreen XL YES Assay Kit

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Number of Data Points

This test provides a total of 96 data points.

The assay can be used to test 4 samples in duplicates in 8 concentrations in order to obtain dose-response curves for the calculation of estrogenic activities, OR to test 32 single-dose samples in duplicates for simplified “yes - no” answers with an estimation of relative activities. This manual describes both versions of the assay which includes all necessary controls, the calculation of EEQ, 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 layouts described in this manual.

Other plate layouts are possible but are not supported by the calculation sheet.

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 receptor hER α . For this purpose the DNA sequences of hER α 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) 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 hER α interacts with the response element on the expression plasmid and modulates the transcription of the lacZ reporter gene. The β -galactosidase is released 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 OD₅₇₀ correlates directly with the amount of secreted β -galactosidase and thus with the activity of the test substance which binds to the estrogen receptor.

The XenoScreen XL YES assay can identify activating (agonistic) activities of test compounds. For the determination of antagonistic activities Xenometrix offers the XenoScreen XL YES/YAS assay (Art. No. N05-233-Y) which detects both estrogenic and androgenic activating and inhibiting activities (agonists and antagonists).

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 uses lyticase and a detergent 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 assay to 18 hours. In addition the accelerated protocol leads also to an enhanced sensitivity.

Assay Description

Growing yeast cells stably transformed with hER α and a β -galactosidase reporter system are exposed to test compound, positive control chemicals (17- β estradiol). 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 OD₆₉₀. The color development is measured at 570 nm and is corrected for diffraction by cells and debris by a simultaneous measurement of OD₆₉₀. The results are evaluated for estrogenic activity, as well as for yeast growth inhibition or cytotoxicity.

Safety Precautions

- The YES 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 chemical 17- β estradiol provided in this kit is a hormonally active substance. 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 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 plastic ware.**
- **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 assay kit contains media and reagents for the analysis of either 4 test compounds in 8 dilutions in duplicates, or for 32 test compounds at 1 concentration in duplicates for agonistic estrogenic endocrine activity. Positive and solvent controls are included.

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

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
- 10× DO medium
- 10× SD medium
- Streptomycin/Ampicillin solution
- DMSO
- Substrate solution CPRG
- 17 β -estradiol positive control
- lyticase
- lacZ lysis buffer
- 2-mercaptoethanol
- 3 96-well plates (2 for the assay, 1 for measurement of cell densities)
- 1 gas-permeable plate sealer
- 2 T25 culture flasks with gas-permeable filter caps

To be ordered separately:

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

Storage Conditions

Each Xenometrix XenoScreen XL YES 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 control E2 before reconstitution (E2)
- 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
- 10x DO medium
- 10x SD medium
- Positive control E2 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 sealer

Required Equipment and Consumables NOT Included with the Kit

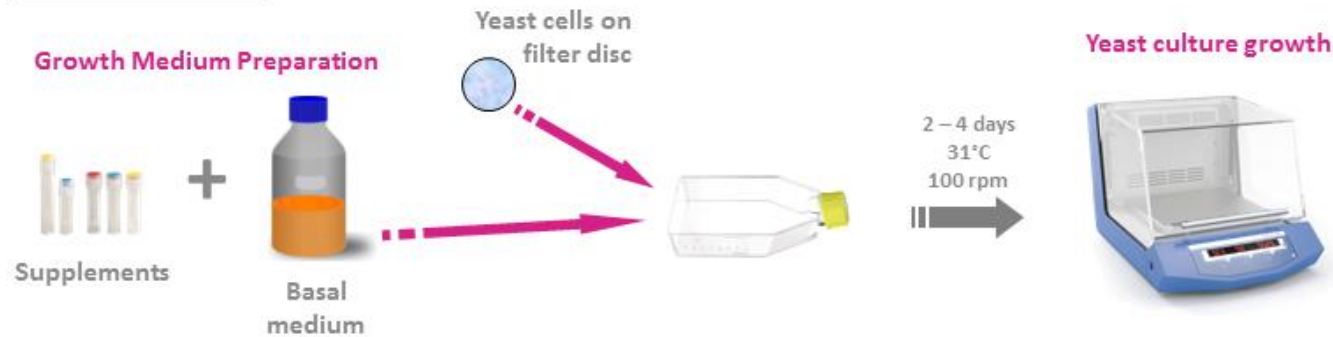
- XenoScreen YES Strain, 1 vial, Art. No. N05-230-E
- Incubator (31°C) with rotating platform (orbital movement)
- High-humidity container (e.g. plastic box with a tight lid, and wet paper tissue)
- Microplate reader capable to read at 570 and 690 nm
- Adjustable micropipettes and sterile tips
(needed volumes: 2, 20–200, 100–1000 μ l)
- Adjustable 8-channel pipettes (needed volumes: 20–200 μ l)
- 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

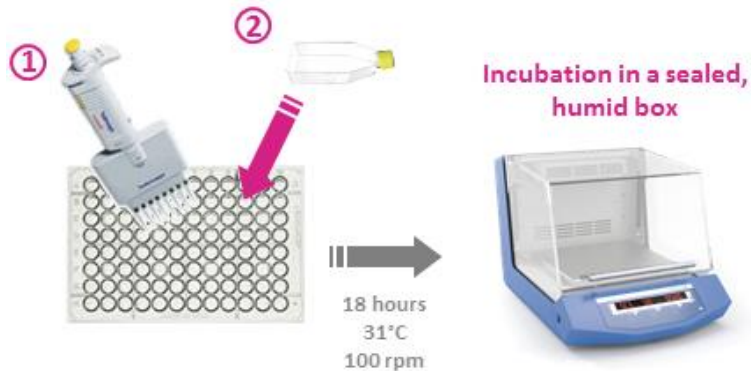
XenoScreen XL – Assay Procedure

Day -4 – Day -2:

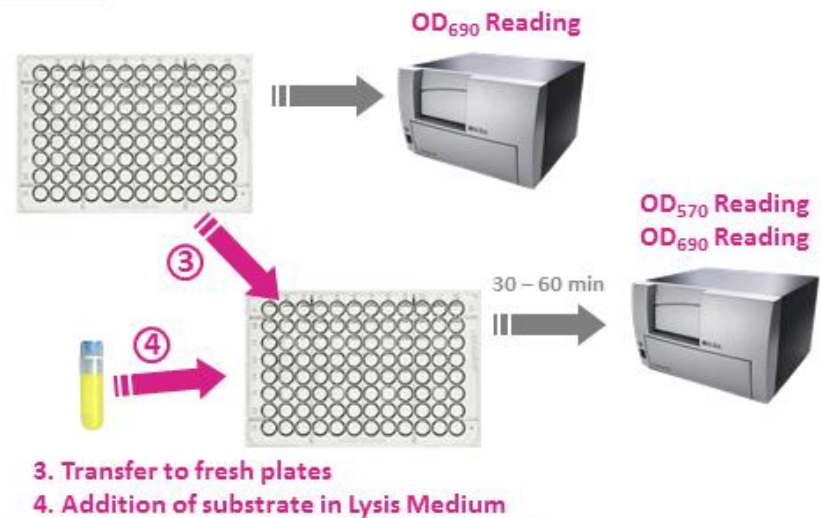


Day 1:

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



Day 2:



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Assay Procedure