



XenoScreen YES/YAS

Yeast-based microplate assay for the detection of compounds with estrogenic and androgenic agonistic and antagonistic activities

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

Short protocol

For 4 x 96 data points

Art. No. N05-233-Y

Upon receipt of your XenoScreen 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

Changelog

Date	New version	Changes
24.4.2014	2.12	<ul style="list-style-type: none">• Changelog added• Revised recommendations for yeast culture preparations (p. 6)• Relaxed OD₆₉₀ requirement for acceptable starting cell density from 0.3 to 0.2 (page 9)• Optimized recommendations for the storage and handling of yeast cells in Appendix A• Changed incubation temperature from 32°C to 31°C to bring in line with XenoScreen XL protocol
22.10.2014		<ul style="list-style-type: none">• More details on regulations for the use of GMO's (Safety Precautions, page 2)• New Xenometrix logo
18.11.2014	3.00	<ul style="list-style-type: none">• Corrected plate layout• Added additional option to send in raw data for evaluation
17.3.2015	3.01	<ul style="list-style-type: none">• 4 T25 flasks instead of 2
8.10.2015	3.02	<ul style="list-style-type: none">• Shelf life of complete growth medium added (page 6)• New order number for Growth Medium
26.10.2015	3.03	<ul style="list-style-type: none">• Specified to add 300 µl of Cu-sulfate to Basal Medium
30.5.2016	3.04	<ul style="list-style-type: none">• Added suggestion to prepare own yeast stocks
20.6.2016	3.05	<ul style="list-style-type: none">• Added Art. Nr. of kit version without cells
5.12.2016	3.06	<ul style="list-style-type: none">• Removed strains from "Kit contents"; yeast cells have to be purchased separately

XenoScreen YES/YAS Assay Kit

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Number of Data Points

This test provides a total of 4 x 96 data points (2x 96 YES and 2x96 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 5 samples for estrogenic, anti-estrogenic, androgenic and anti-androgenic activities with all necessary controls, the calculation of EEQ and AEQ.

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 hER α and hAR. For this purpose the DNA sequences of hER α 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; 241-248; Sohoni, P. and Sumpter, J.P. 1998. J. Endocrinol. 158, 327-339)

Upon binding of a ligand, the hER α 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 OD₅₇₀ 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 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 5 α -dihydrotestosterone for YAS). Inhibition of the response relative to this fixed agonist concentration is a sign of antagonist activity.

The assay also identifies cytotoxic effects which lead to growth arrest or lysis of the tester yeast cells. Cytotoxicity is measured as a reduction of light scatter at a wavelength of 690 nm.

Assay Description

Growing yeast cells stably transformed with either hER α (YES) or hAR (YAS) are exposed to serial dilutions of test compound, positive control chemicals (17- β estradiol for YES and 5 α -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 48 hrs at 31°C in the presence of a substrate for β -galactosidase. The expression of β -galactosidase is directly linked to the presence of activating (agonistic) or inhibiting (antagonistic) compounds and leads to a conversion of the yellow substrate to the red cleavage product chlorophenol red. Color development and yeast growth are quantified in a photospectrometer at 570 and 690 nm, respectively. 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 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 YES/YAS assay kit contains media and reagents for the analysis of 5 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 separately: 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
- Substrate solution CPRG
- 1 vial with 17 β -estradiol positive control ("E2", red cap; YES)
- 1 vial with 5 α -dihydrotestosterone positive control ("DHT", blue cap; YAS)
- 1 vial with 4-hydroxytamoxifen control antagonist ("HT", yellow cap; YES)
- 1 vial with flutamide control antagonist ("FL", green cap; YAS)
- 1 vial with DMSO solvent
- 5 96-well plates (4 assay plates + 1 dilution plate)
- 4 gas-permeable plate sealers
- 4 T25 culture flasks with gas-permeable filter cap

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 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)
- *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
- 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
- 96-well plates
- T25 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: 10, 20-200, 100-1000 µl)
- Adjustable 8-channel pipettes (needed volumes: 2 µl, 23 µl, 50 µl and 100 µl)
- Serological pipettes (sterile)
- Pipetting reservoirs (sterile)
- Gloves

Recommended:

- Inverted microscope to inspect yeast cultures

Important Note on Equipment Needed

The standard version of this assay requires a multichannel pipette with a 2 µl capacity for the transfer of standard and sample dilutions from the dilution plate to the assay plates. If such a pipette is not available in your lab you may perform the assay with some slight modifications using a multichannel pipette with 20 µl capacity instead.

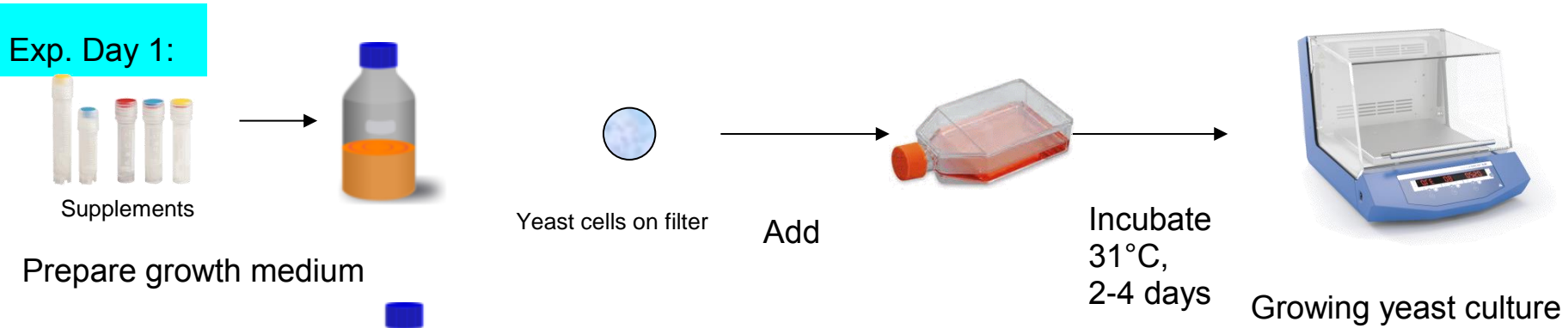
The changes are in the preparation of the assay plates with assay medium and antagonist assay medium, and the preparation of an additional dilution plate (96-well plate, not provided in the kit).

The modifications are directly described in the text using red italics.

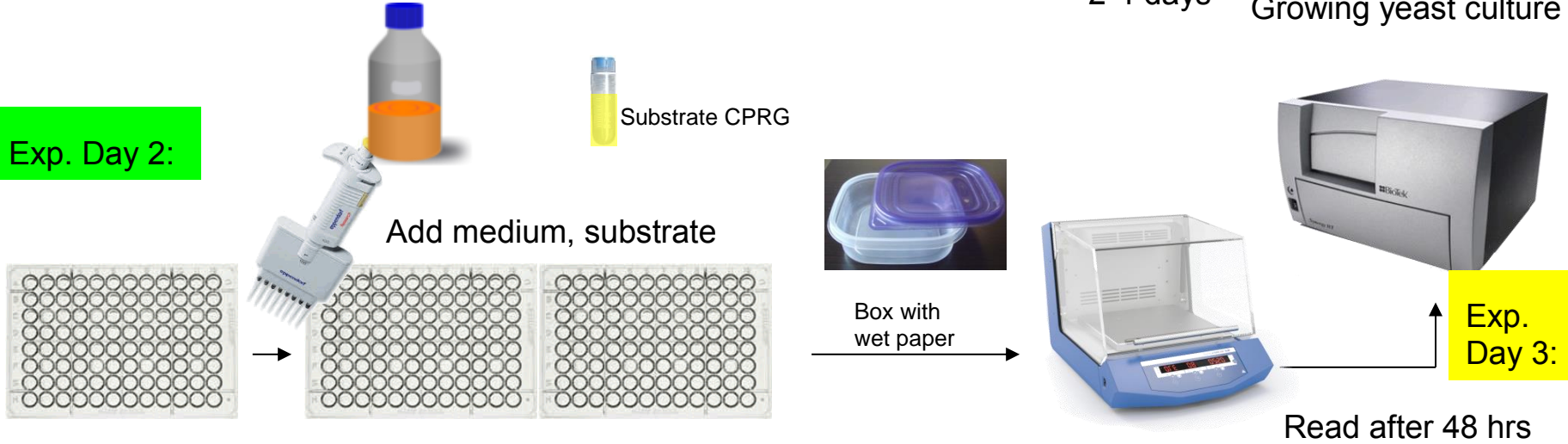
XenoScreen Procedure



Exp. Day 1:



Exp. Day 2:



Prepare dilutions (samples, controls)

Transfer dilutions to assay plates, add yeast cells

Seal, incubate in humid box, incubate at 31°C for 48 hr

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