Testing of estrogenic and androgenic reference compounds using 2 versions of the yeast-based YES and YAS assay

Markus Kamber
Xenometrix AG, Gewerbestr. 25, CH - 4123 Allschwil, Switzerland

Abstract:

The performance of 2 commercially available kits for the detection of estrogenic and androgenic compounds was evaluated. The kits ("XenoScreen" and "XenoScreen XL", Xenometrix AG, Switzerland) are based on yeast cells transfected with the human estrogen and androgen receptors, respectively (YES and YAS, Routledge and Sumpter, 1996). The tests are performed in microtiter plates and are able to detect both activating (agonist) and inhibiting (antagonist) activities. Hormonal activities of test substances are detected using a lacZ reporter gene construct (β-galactosidase) and the highly sensitive substrate CPRG leading to a color change in the medium from yellow to purple. The XenoScreen kit is based on the original protocol and uses an incubation time of 48 hrs, whereas the XenoScreen XL kit is based on a modified 18-hr exposure protocol using the enzyme lyticase to facilitate the release of the β-galactosidase from the yeast cells.

Several chemicals with known estrogenic and androgenic activities ranging from strong to weak as well as negative control compounds were evaluated, including 14 chemicals recommended in the OECD 455 guideline "Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists" for the testing of laboratory proficiency. Both kit versions were able to correctly identify these chemicals.

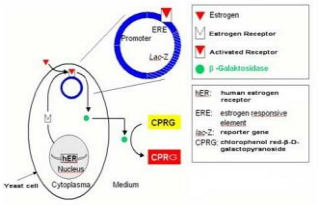
The results demonstrate the usefulness of the 2 YES/YAS kits to correctly identify compounds with known estrogenic and androgenic agonist and antagonist activities.

Introduction :

The 2 test kits address the need to identify rapidly and reliably activating (agonistic) and inhibiting (antagonistic) estrogenic and androgenic activities in environmental (aqueous) samples, or of chemical , pharmaceutical or cosmetic compounds and mixtures. Both kits are based on yeast cells (*Saccharomyces cerevisiae*) into which the human estrogen or androgen receptors and an lacZ reporter gene system were integrated. Hormonal activity is detected in a microplate format, colorimetric readout *Routledge, E.J. and Sumpter, J.P. 1996. Environ.Toxicol. Chem. 13; 241-248*

Methods:

XenoScreen and XenoScreen XL are based on human estrogen and androgen receptors integrated into the chromosome of the yeast *Saccharomyces cerevisiae* which also harbor an expression vector for lacZ (β-galactosidase). Binding to the receptor leads to the production of β-galactosidase which converts the yellow substrate CPRG into a purple product.

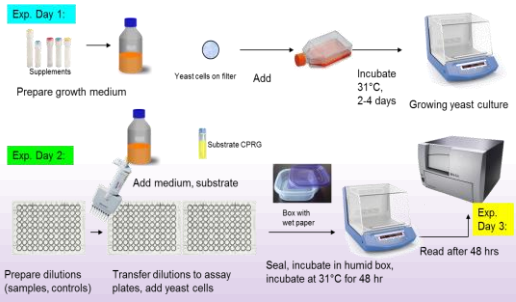


Both assay versions can measure agonistic and antagonistic activities of test compounds. Inhibiting activities are detected in the presence of a sub-maximal, constant amount of an agonist.

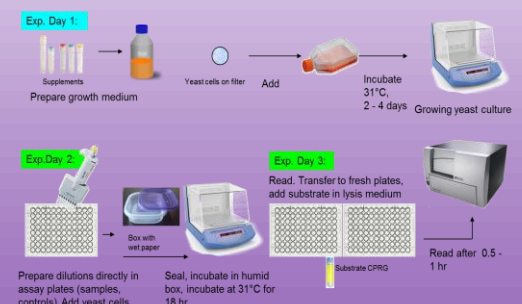
XenoScreen XL YES/YAS uses lyticase and a detergent to facilitate the release of β-galactosidase into the medium which allows for a shorter exposure time of 18 hrs vs. 48 hrs in the standard XenoScreen YES/YAS and also results in an improved sensitivity (see "Results"). The XL version uses a concentrated test medium which allows to test aqueous samples with minimal dilution (1:1.5).

The kits include the yeast cells and all necessary media, reagents and positive controls, as well as microtiter plates and plate sealers. Results can be compiled, graphed and evaluated with the included Excel worksheet.

Procedure XenoScreen



Procedure XenoScreen XL



Results:

Proficiency Chemicals: Estrogen Agonists and Antagonists

Name	CAS #	Class	Expected response [1]	Highest tested conc. (M^{-1})	XenoScreen EC50 (M^{-1})	XenoScreen EC10 (M^{-1})	XenoScreen XL EC50 (M^{-1})	XenoScreen XL EC10 (M^{-1})	Remarks
Agonists									
17β-Estradiol	50-28-2	Steroid	positive	1.0 x 10-8	1.4 x 10-10	4.9 x 10-11	5.0 x 10-11	1.4 x 10-11	
Diethylstilbestrol	56-53-1	Cyclic Hydrocarbon	positive	2.5 x 10-8	4.2 x 10-10	2.2 x 10-10	1.6 x 10-10	4.7 x 10-11	
17α-Estradiol	57-91-0	Steroid	positive	1.0 x 10-7	1.1 x 10-8	3.6 x 10-9	1.6 x 10-9	5.1 x 10-10	
meso-Hexestrol	84-16-2	Cyclic Hydrocarbon, Phenol	positive	1.0 x 10-7	2.9 x 10-10	8.0 x 10-11	1.4 x 10-10	2.9 x 10-11	
4-tert-Octylphenol	140-66-9	Phenol	positive	1.0 x 10-5	3.3 x 10-6	1.7 x 10-6	2.6 x 10-6	7.8 x 10-7	
Genistein	446-72-0	Flavonoid, Heterocyclic Cpd.	positive	1.0 x 10-5	3.1 x 10-6	1.1 x 10-6	1.8 x 10-7	5.7 x 10-8	
Bisphenol A	80-05-7	Phenol	positive	1.0 x 10-4	5.1 x 10-6	1.4 x 10-6	2.8 x 10-6	7.8 x 10-7	
Kaempferol	520-18-3	Flavonoid, Heterocyclic Cpd.	positive	1.0 x 10-4	2.9 x 10-5	1.5 x 10-5	7.1 x 10-5	2.0 x 10-5	
Butylbenzyl phthalat	85-68-7	Carocyclic acid, Ester, Phthalic acid	positive	1.0 x 10-4	1.3 x 10-4	4.5 x 10-5	1.3 x 10-5	3.9 x 10-6	
p,p'-Methoxychlor	72-43-5	Halogenated Hydrocarbon	positive	1.0 x 10-3	8.8 x 10-5	2.4 x 10-5	1.9 x 10-5	5.1 x 10-6	
Ethyl paraben	120-47-8	Carboxylic acid, Phenol	positive	1.0 x 10-3	1.6 x 10-4	4.4 x 10-5	1.9 x 10-5	5.1 x 10-6	
Atrazine	1912-24-9	Heterocyclic cpd.	negative	1.0 x 10-3	inactive	inactive	inactive	inactive	
Spironolactone	52-01-7	Lactone, Steroid	negative	1.0 x 10-3	inactive	inactive	inactive	inactive	
Ketoconazole	65277-42-1	Heterocyclic cpd.	negative	1.0 x 10-3	inactive	inactive	inactive	inactive	toxic >1 x 10-6
Reserpine	50-55-5	Heterocyclic cpd., Indole	negative	1.0 x 10-3	inactive	inactive	inactive	inactive	
Antagonists [2]									
4-Hydroxytamoxifen	68047-06-3	Cyclic Hydrocarbon	positive	1.0 x 10-5	1.3 x 10-7		2.7 x 10-6		

[1] from "OECD GUIDELINE 455 FOR THE TESTING OF CHEMICALS:
Stably Transfected Human Estrogen Receptor-α Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals
and from ICCVAM (2003) ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors:
Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays
[2] Only EC50 values are given for antagonists, smaller inhibition values are bound to give false positves

Proficiency Chemicals: Androgen Agonists and Antagonists

Name	CAS #	Class	Expected response [1]	Highest tested conc. (M^{-1})	XenoScreen EC50 (M^{-1})	XenoScreen EC10 (M^{-1})	XenoScreen XL EC50 (M^{-1})	XenoScreen XL EC10 (M^{-1})	Remarks
Agonists									
5α-Dihydrotestosterone	521-18-6	Steroid, nonphenolic	positive	1 x 10-6	4.3 x 10-9	6.5 x 10-10	2.0 x 10-9	4.7 x 10-10	
Dexamethasone	50-02-2	Steroid, nonphenolic	positive	1 x 10-3	>1 x 10-3	>1 x 10-3	1.4 x 10-3	3.1 x 10-4	
Medroxyprogesteroneacetate	71-58-9	Steroid, nonphenolic; Polycyclic hydrocarbon	positive	1 x 10-6	2.9 x 10-8	4.7 x 10-9	1.5 x 10-8	2.9 x 10-9	
Testosterone	58-22-0	Steroid, nonphenolic	positive	1 x 10-5	4.5 x 10-9	7.5 x 10-10	2.9 x 10-9	5.6 x 10-10	
4-Androstenedione	63-05-8	Steroid, nonphenolic	positive	1 x 10-5	4.2 x 10-7	1.4 x 10-8	1.2 x 10-8	3.0 x 10-9	
Spironolactone	52-01-7	Steroid, nonphenolic; Pregnene lactone	positive	1 x 10-3	1.4 x 10-5	1.3 x 10-6	2.6 x 10-6	6.7 x 10-7	
Progesterone	57-83-0	Steroid, nonphenolic; Pregnenedione	positive	1 x 10-5	4.6 x 10-8	8.2 x 10-9	8.9 x 10-9	2.2 x 10-9	
17α-Methyltestosterone	58-18-4	Steroid, nonphenolic; Androstene	positive	1 x 10-7	9.4 x 10-9	1.7 x 10-9	4.6 x 10-9	8.9 x 10-10	
Flutamide	13311-84-7	Amide; Anilide; Nitrobenzene	negative	1 x 10-4	inactive	inactive	inactive	inactive	
4-*tert*-Octylphenol	140-66-9	Alkylphenol; Phenol	negative	1 x 10-3	inactive	inactive	inactive	inactive	toxic > 1 x 10-5
Bisphenol A	80-05-7	Diphenylalkane; Bisphenol; Phenol	negative	1 x 10-3	inactive	inactive	inactive	inactive	
Di(2-ethylhexyl)phthalate	117-81-7	Phthalate	negative	1 x 10-3	inactive	inactive	inactive	inactive	
Antagonists [2]									
Hydroxyflutamide	52806-53-8	Amide; Anilide; Nitrobenzene	positive	1 x 10-4	1 x 10-5	-	6 x 10-6	-	
Bisphenol A	80-05-7	Diphenylalkane; Bisphenol; Phenol	positive	1 x 10-3	3 x 10-4	-	4 x 10-5	-	
Flutamide	13311-84-7	Amide; Anilide; Nitrobenzene	positive	1 x 10-4	2.0 x 10-5	-	1.2 x 10-5	-	
Spironolactone	52-01-7	Steroid, nonphenolic; Pregnene lactone	positive	1 x 10-3	>1 x 10-3, bi-phasic	-	inactive	-	Agonist
Procymidone	32809-16-8	Organochlorine; Cyclic imide	positive	1 x 10-3	5.0 x10-6	-	2.0 x 10-6	-	
Progesterone	57-83-0	Steroid, nonphenolic; Pregnenedione	positive	1 x 10-5	inactive	-	inactive	-	Strong agonist
Vinclozoline	50471-44-8	Organochlorine; Cyclic imide; Carbamate	positive	1 x 10-3	2.8 x 10-5	-	9 x 10-6	-	
Medroxyprogesteroneacetate	71-58-9	Steroid, nonphenolic; Polycyclic hydrocarbon	negative	1 x 10-6	inactive	-	inactive	-	

[1] from "(Draft) OECD GUIDELINE FOR THE TESTING OF CHEMICALS
Stably Transfected Human Androgen Receptor-α Transcriptional Activation Assay for Detection of Androgenic Agonist Agonist/Antagonist Activity of Chemicals (Version 2010 Nov. 25)
and from ICCVAM (2003) ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors:
Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays
[2] Only EC50 values are given for antagonists, smaller inhibition values are bound to give false positves

Limits of Detection LoD: Definition: LoD = Mean of Solvent Control + 3x SD

17β-Estradiol: XenoScreen: 1.8×10^{-11} M; XenoScreen XL: 4.2×10^{-12} M

Dihydrotestosterone: XenoScreen: 4.0×10^{-10} M; XenoScreen XL: 2.1×10^{-10} M

Conclusions:

Both versions of the XenoScreen assay (standard and XL) are able to correctly identify a series of strong to weak estrogenic and androgenic proficiency chemicals. Similarly, compounds expected to be negative based on published data are also correctly classified as negative, demonstrating the specificity of the assay.

Both activating and inhibiting activities can be measured. The 2 only exceptions in the YAS antagonist assay can be explained by dominance of of the agonistic effect in dual-mode compounds.

www.xenometrix.fr
www.xenometrix.ch