Evaluation of Alternative Estrogen Activity Assays for New Computational Models to Support the Endocrine Disruption Screening Program

Parisa Sadrpour[1,2], Scott G. Lynn[3], Richard S. Judson[2], Steven O. Simmons[2]

[1]Oak Ridge Institute for Science Education, [2]Office of Research and Development, [3]Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency.

sadrpour.parisa@epa.gov

Abstract ID
4222/P771

Introduction

- The Endocrine Disruption Screening Program (EDSP) was launched by EPA to screen chemicals for perturbation of the estrogen, androgen, and thyroid pathways in humans and wildlife.
- Recent work has sought to identify replacement strategies for several animal-based tests in the EDSP assay battery.
- A computational model to predict estrogen receptor (ER) activity was developed using 18 ToxCast assays and has been proposed as an alternative for three current EDSP Tier 1 tests, including the in vivo uterotrophic assay[1].
- Subsequent analyses revealed that as few as four high quality assays could provide comparable predictivity to the original model, provided those assays covered the key events in ER pathway activation: (1) ligand binding, (2) dimerization, (3) transcriptional activation, and (4) proliferation[2].
- Several of the original ToxCast assays used to build the streamlined ER models are no longer available or have practical barriers to wide-scale use including the top-performing transactivation (ATG_ERa_TRANS; Attagene) and dimerization (OT_ERa_ERb; Odessey Thera) assays.

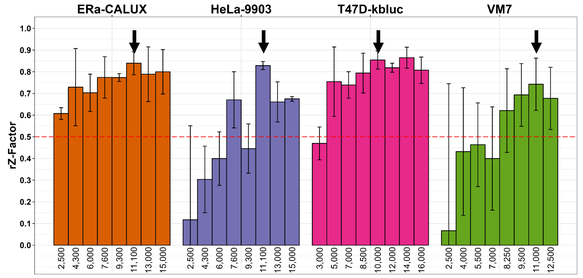
Objectives

Assay	Key Event	Cell Line	Source
ERa-CALUX	Transactivation	U2OS	BioDetection Systems
HeLa-9903	Transactivation	HeLa	JCBR Cell Bank
T47D-kbluc	Transactivation	T47D	US EPA
VM7Luc	Transactivation	MCF7	University of California-Davis
ER12	Dimerization	HepG2	US EPA

- Optimize five alternative ER assays to maximize performance in a 384-well high-throughout screening format.
- Test 33 reference chemicals listed in OECD Test Guideline (TG) 455 in concentration response.
- Use the receiver operator characteristic (ROC) to establish optimized activity thresholds for each assay to properly classify positive and negative reference chemicals.
- Fit nonlinear regression models to determine half-maximal activity concentration (AC_{50}) values for comparison of legacy assays to candidate replacement assays.

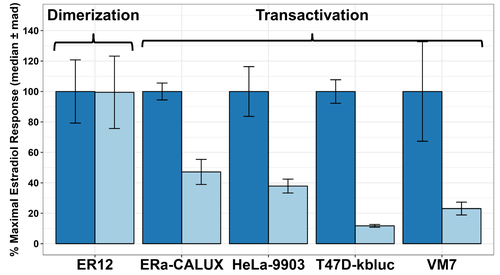
Assay Optimization

Seeding Density



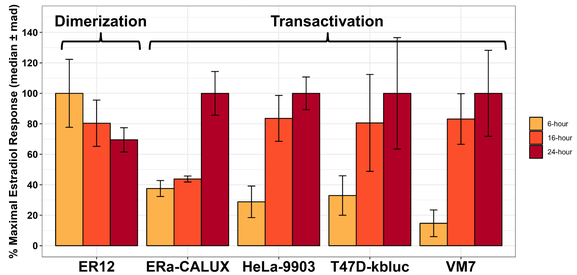
Optimal seeding densities were identified for each transactivation assay (black arrows) using rZ-factors (y-axis). Cells were treated with DMSO (0.5% final) or 17β-estradiol up to 998 pM (final) for 24 hours. rZ-factors were calculated using the responses of estrogen- and DMSO-treated wells at each seeding density (x-axis). The 0.5 minimal threshold (dotted red line) is required for high-throughput screening (HTS) applications.

Medium Formulation



ER transactivation assays performed better using high FBS medium while serum concentration had no effect on ER dimerization assay performance. Assay performance was assessed using media containing high (10%) or low (2%) charcoal-dextran stripped FBS. Cells were treated with DMSO (0.5% final) or 17β-estradiol for 24 hours.

Exposure Time



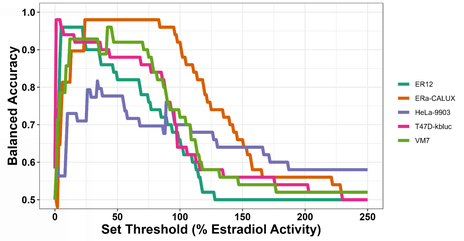
ER dimerization assay performance diminished with increased exposure time while each transactivation assay saw increased assay performance at 24 hours compared to shorter exposure durations. Cells were treated with DMSO (0.5% final) or 17β-estradiol for 6, 16, or 24 hours.

Pre-Validation

Chemicals

Reference Chemical	Known ER Bioactivity
17a-Estradiol	Positive
17a-Ethinyl Estradiol	Positive
17b-Estradiol	Positive
17b-Trenbolone	Positive
17-Methyltestosterone	Positive
19-Nortestosterone	Positive
4-Cumylphenol	Positive
4-tert-Octylphenol	Positive
Apigenin	Positive
Benzyl butyl phthalate	Positive
Bisphenol A	Positive
Bisphenol B	Positive
Coumestrol	Positive
Diadzein	Positive
Dibutyl phthalate	Positive
Diethylstilbestrol	Positive
Estrone	Positive
Ethyl 4-hydroxybenzoate	Positive
Genistein	Positive
Kaempferol	Positive
Kepone	Positive
meso-Hexestrol	Positive
Methoxychlor	Positive
Morin	Positive
Norethynodrel	Positive
Testosterone	Positive
Atrazine	Negative
Corticosterone	Negative
Haloperidol	Negative
Ketoconazole	Negative
Linuron	Negative
Reserpine	Negative
Spironolactone	Negative

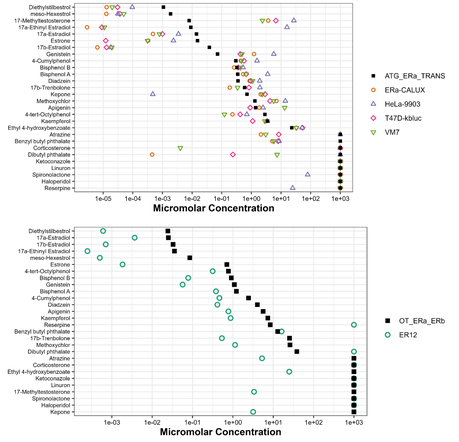
Threshold Optimization



Threshold optimization maximized predictivity to 95+% balanced accuracy for each assay except HeLa-9903 (82%). Reference chemicals data were scaled to vehicle (0%) and 17β-estradiol (100%). The receiver operator characteristic was used to identify optimal assay activity thresholds to discriminate positive reference chemicals from negatives. The resulting balanced accuracy (y-axis) is plotted for each threshold (x-axis).

Most active reference chemicals have equal or lower AC_{50} values in the candidate ER transactivation and ER dimerization assays compared to the Attagene (ATG) and Odyssey Thera (OT) assays, respectively. AC_{50} values (x-axis) for 28 reference chemicals previously tested in ToxCast are shown.

Potency Comparison to Legacy Assays



Conclusions and Future Directions

- After optimization, all five assays proved amenable to high-throughput screening with rZ-factors well above 0.5.
- The optimal medium formulation and exposure time for the ER12 dimerization assay was distinct from the pattern observed for the transactivation assays.
- ROC analysis identified optimal thresholds that maximized balanced accuracy for each assay.
- The candidate assays produced equivalent or lower AC_{50} values compared to the legacy assays for most chemicals.
- Each assay is currently being used to screen a larger 237-chemical sample set composed of both ER and androgen receptor reference chemicals.
- These data will be used to determine whether high predictivity can be achieved using these refined, widely available, and transferable assays in an updated ER computational model.

References:

1. Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High-Throughput Screening Assays for the Estrogen Receptor; Judson et. al., Toxicological Sciences, 2015 Nov;148(1):137-54. doi: 10.1093/toxsci/kfv168.
2. On selecting a minimal set of in vitro assays to reliably determine estrogen agonist activity; Judson et. al., Regulatory Toxicology and Pharmacology, 2017 Dec:91:39-49. doi: 10.1016/j.yrtph.2017.09.022.

Alternative Estrogen Receptor assays improve the sensitivity and transferability of the ER computational model

U.S. Environmental Protection Agency
Office of Research and Development