In Vitro Estrogen Receptor Binding Assay (ERB)

Need

Endocrine disruption is of regulatory importance globally and is a primary consideration in determining the safety and/or efficacy of a dermatological drugs, cosmetics, chemicals, and agrochemicals. The estrogenic pathway involves binding of estrogens with estrogen receptors (ERs) that subsequently affect transcription of estrogen-controlled gene expression. Compound or chemical perturbation of the ability of estrogen to bind to the ER may have adverse effects on normal development, reproductive health, and/or the integrity of the reproductive system. For new and existing drugs, cosmetics, chemicals, and agrochemicals, the cost and time necessary to assess disruption of the ability for estrogen to bind the ER *in vivo* is prohibitory to efficient product development and safety assessment. This ligand-receptor interaction is the initial step of the estrogen signaling pathway and is critical for normal function of the estrogen hormone pathway.

Solution

The potential for a compound or chemical to bind to the estrogen receptor can be assessed early in the product development cycle to determine their relative risks for causing endocrine disruption. In our Estrogen Receptor Binding Assay, the PolarScreen™ ER Alpha Competitor Assay is used. This assay uses a full-length, native (untagged) alpha isoform of the human estrogen receptor and a novel, high-affinity, fluorescent estrogen ligand (Fluormone ES2 Green) in a homogenous, mix-and-read assay format. Full length ER alpha is added to a fluorescent estrogen ligand to form an ER-Fluormone ES2 Green complex. This complex is then incubated with various concentrations of the test compound(s). Fluorescence polarization is then assessed to determine compound binding affinity to the ER. Competing test compounds will displace the Fluormone ES2 Green ligand from the ER, permitting it to tumble rapidly and resulting in low polarization values. A shift in the fluorescence polarization value in the presence of test compound is used to determine the relative affinity of test compounds for the ER. Data are modeled using GraphPad Prism to determine the IC50 for each test article, if possible.

Accurate & reliable data

Fast turnaround times

Unsurpassed expertise

Collaborative approach

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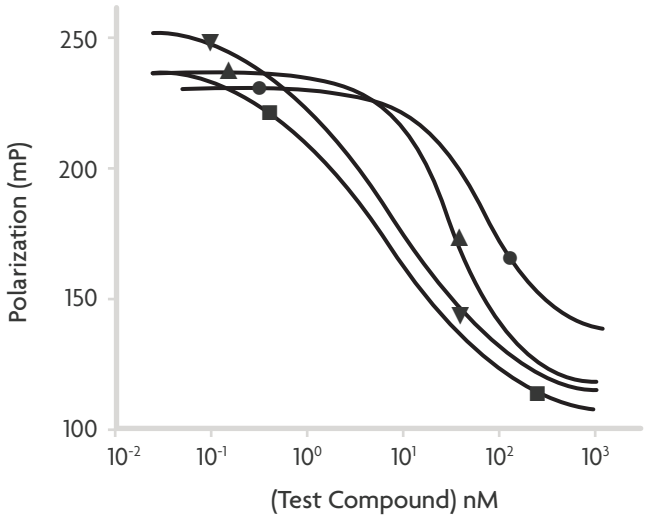
Standard Protocol

ASSAY PARAMETERS	PROTOCOL
Model	PolarScreen™ ER Alpha Competitor Assay
Replicates	3
Solvent of Choice	DMSO (preferred) or assay buffer
Test Article Formulation	300, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, and 0.001 µM (depending on solubility; may be adjusted based on binding capacity)
Solvent Controls	DMSO (or assay buffer)
oPositive Control	17β-Estradiol (100, 30, 10, 3, 1, 0.3, 0.1, and 0.03 nM)
Reference Controls	Norethindrone and Octyltriethoxysilane
Exposure Time	6 hours
ER Binding	Fluorescence polarization
Time to Complete	3-5 weeks from test article receipt
Regulatory	Non-GLP or GLP
Deliverables	Full Report, Binding affinity (IC50 values, if possible), categorization (binder or non-binder)

Example Data



[Test Compound] nM	IC_{50}(nM)
■ DES	5.4
▲ 4-OH Tamoxifen	29.6
▼ Estradiol	5.7
● Tamoxifen	60.9

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