In Vitro Androgen Receptor Binding Assay (ARB)

Need

Endocrine disruption is a primary consideration in determining the safety and/or efficacy of a dermatological drugs, cosmetics, chemicals, and agrochemicals. The androgenic pathway involves binding of androgens with androgen receptors (ARs) that subsequently affect transcription of androgen-controlled gene expression. Compound or chemical perturbation of the ability of androgen to bind the AR 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 androgen to bind the AR *in vivo* is prohibitory to efficient product development and safety assessment.

Solution

LifeNet Health LifeSciences offers the AR binding assay for the assessment of potential interference in the normal androgen hormone pathway. The AR binding assay provides mechanistic insight rapidly, which can be used for screening and prioritization purposes

In our ARB assay, the PolarScreen™ Androgen Receptor Competitor Assay is used; this assay uses a rat AR-ligand binding domain tagged with glutathione-S-transferase (GST) and a novel, high-affinity, fluorescent androgen ligand (Fluormone™ AL Green) in a homogenous, mix-and-read assay format. The AR is added to a fluorescent androgen ligand to form an AR-Fluormone™ AL 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 AR. Competing test compounds will displace the Fluormone™ AL Green ligand from the AR, 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 AR. 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

LifeNet Health® LifeSciences

Talk with one of our experts for help with general inquiries, protocol details, or becoming a new client.

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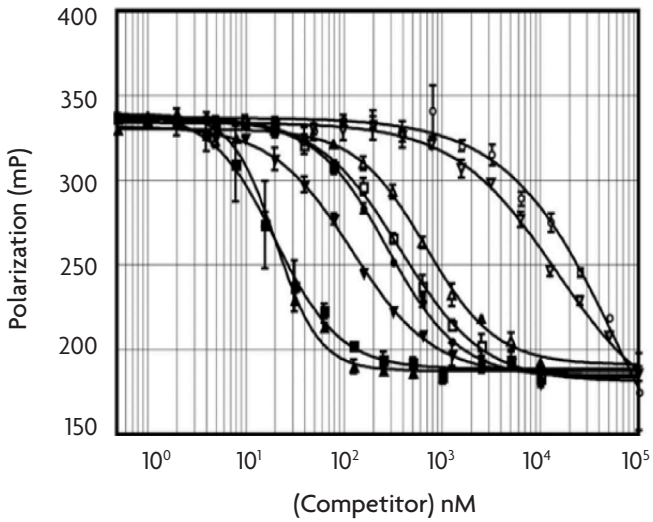
Standard Protocol

ASSAY PARAMETERS	PROTOCOL
Model	PolarScreen™ Androgen Receptor 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)
Positive Control	Methyltrienolone (R1881; 300, 100, 30, 10, 3, 1, 0.3, and 0.1 nM)
Reference Controls	Dexamethasone 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 including: Binding affinity (IC_{50} values, if possible), categorization (binder or non-binder)

Example Data



[Test Compund] nM	IC_{50}(nM)
■ Testosterone	20
▲ DHT	20
▼ Estradiol	120
● Cyproterone Acetate	277
□ Progesterone	371
△ Hydroxyflutamide	679
▽ Flutamide	>105
○ DEX	>105

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