In Vitro Estrogen Receptor Transcriptional Activation Assay (ERTA)

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. One of the pathways, the estrogenic pathway, involves interaction of estrogens with estrogen receptors (ERs) that subsequently affects transcription of estrogen-controlled gene expression. Compound or chemical perturbation of normal estrogenic transcriptional activation pathways 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 estrogenic transcriptional activation pathways in vivo is prohibitory to efficient product development and safety assessment. Fortunately, *in vitro* methods exist for the endocrine disruption potential of drugs, cosmetics, chemicals, and agrochemicals, which provide mechanistic insight rapidly, which can be used for screening and prioritization purposes.

Solution

LifeNet Health LifeSciences offers the validated ERTA assay for the assessment of potential estrogenic activation of test compounds or chemicals, operating in full compliance with the OECD 455 guideline. In the ERTA assay at LifeNet Health LifeSciences, the hERα-HeLa-9903 cell line is used. This cell line has two stably inserted constructs; the hERα expression construct (encoding the full-length human receptor), and a firefly luciferase reporter construct containing five tandem repeats of a vitellogenin Estrogen-Responsive Element (ERE). Upon ligand binding, the ERs translocate to the nucleus where they interact with the ERE on the on the firefly luciferase construct, resulting in expression of the firefly luciferase enzyme. This enzyme transforms the luciferin substrate to a bioluminescent product that can be quantitatively measured with a plate reader. Using the basic procedures outlined below, the luminescent signal (i.e., ER transcriptional activation) and cell viability are determined immediately following exposure to test chemicals or compounds. This assay is performed for both agonism (activating ER specific gene expression) and antagonism (inhibiting ER specific gene expression). At least two runs will be conducted for both the agonism and the antagonism assays to determine if the test article(s) are positive or negative for ER transactivation (Tables 1 and 2).

Accurate & reliable data

Fast turnaround times

Unsurpassed expertise

Collaborative approach

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Standard Protocol

ASSAY PARAMETERS	PROTOCOL
Model	hERα-HeLa-9903 cell line
Replicates	3
Solvent of Choice	DMSO (preferred), sterile water, culture medium, or ethanol
Test Article Formulation	1 mM, 100 µM, 10 µM, 1µM, 100 nM,10 nM, 1 nM, 100 pM, and 10 pM (depending on solubility and cytotoxicity pre-testing)
Solvent Controls	DMSO (or sterile water)
Agonism Positive Control	1 nM 17β-Estradiol
Agonism Reference Controls	17β-estradiol, 17β-estradiol, 17β-methyltestosterone and corticosterone
Antagonism Positive Control	10 µM Tamoxifen
Antagonism Reference Controls	Tamoxifen and Flutamide
Exposure Time	24 ± 2 hours
Cell Viability Assessment	MTT assay
ERTA Induction / Inhibition	Luminescence assay
Time to Complete	4-6 weeks from test article receipt
Regulatory	Non-GLP or GLP
Deliverables	Full Report, Agonism (PC_{10}, PC_{50} and RPC_{max}), Antagonism (IC_{30} and IC_{50})

Key References
OECD (2021), Test No. 455: Performance-Based Test Guideline for Stably Transfected Transactivation *In Vitro* Assays to Detect Estrogen Receptor Agonists and Antagonists, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264076372-en.

Table 1. ER agonist

Positive	If the RPC_{Max} is obtained that is equal to or exceeds 10% of the response of the positive control in at least two of two or two of three runs.
Negative	If the RPC_{Max} fails to achieve at least 10% of the response of the positive control in two of two or two of three runs.

Table 2. ER antagonist

Positive	If the IC_{30} is calculated in at least two of two or two of three runs.
Negative	If the IC_{30} fails to calculate in two of two or two of three runs..

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