CALUX®
Highlight

BDS
BioDetection Systems

ER CALUX®

The estrogen responsive (ER) CALUX consists of the human breast cancer cell line T47D, incorporating the firefly luciferase gene coupled to estrogen responsive elements (EREs) as a reporter gene for the presence of estrogens and estrogen-like compounds. Following binding of these compounds to the intracellular estrogen receptors (alpha and beta), the ligand-receptor complex binds the ERE. This will lead to expression of proteins that are under normal circumstances associated to ERE-mediated transcription, but also luciferase. After addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor activation, which is benchmarked against the relevant reference compound 17β-estradiol (E2), and expressed as toxic equivalents (TEQs), or bioanalytical equivalents (BEQs).

Specification	ER CALUX
Basal cell line	T47D
Species	human
Tissue	breast
Positive control	17β-estradiol
Endpoint (pure compounds)	EC or PC concentration, lowest effect concentration (e.g. PC10)
Endpoint (mixtures)	Toxic equivalents in pg TEQ/g sample processed
Test duration	24hr (incubation time)
Specificity	Binding to the endogenous estrogen receptors. Ligand selections can be made through compound class selective workup methods and/or metabolic modules.
Assay interferences	Minimal because of use of highly pathway specific construct, and extensive QA/QC. Due to the use of endogenously expressed receptors cross-talk may occur. Cytotoxicity and non-specific luciferase interferences experienced with certain ligands and samples can being assessed with the cytotox CALUX assay.
Sensitivity (LOD/Q)	Typically in the low pg range (matrix- and sample size-dependent)
Matrices	Any type of sample
Sample volume/mass	Matrix- and desired limit of quantification (LOQ)-dependent
Amount of compound	Typically 10 mg. Much lower for high potency compound provided in DMSO
Assessment criteria	In house methods, compliant with relevant application/regulations.
SOPs and Guidelines	BDS internal, Dutch Rijkswaterstaat RIKZ-Specie-08 guideline; ISO 19040-3.
HTS protocol	Not available
Key reference	Legler, J., Van den Brink, C.E., Brouwer, A., Murk, A.J., Van der Saag, P.T., Vethaak, A.D., and Van der Burg, B. (1999) Development of a stably transfected estrogen receptor-mediated luciferase reporter gene assay in the human T47-D breast cancer cell line. Toxicological Sciences 48, 55-66.