

ERalpha CALUX®

The estrogen receptor alpha responsive (ERalpha) CALUX consists of the human osteosarcoma cell line U2OS, incorporating the firefly luciferase gene coupled to estrogen responsive elements (EREs) as a reporter gene for the presence of estrogens and estrogen-like compounds, as well as human ERalpha. Following binding of these compounds to the intracellular ERalpha, 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 17 β -estradiol (E2), and expressed as toxic equivalents (TEQs), or bioanalytical equivalents (BEQs).

Specification	ERalpha CALUX
Basal cell line	U2OS
Species	human
Tissue	bone
Positive control	17 β -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 ERalpha only. 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 specific receptor construct, and extensive QA/QC. Cytotoxicity and non-specific luciferase interferences experienced with certain ligands and samples can be 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 and ISO 19040-3 (water/waste water); OECD TG455; Dutch Rijkswaterstaat RIKZ-Specie-08 guideline; Australian Water Commission; EPA California.
HTS protocol	BDS; see EURL-ECVAM DB-ALM Protocol n° 197 : Automated CALUX reporter gene assay procedure
Key reference	Sonneveld, E., Jansen, H.J., Riteco, J.A.C., Brouwer, A., Van der Burg, B. (2005) Development of androgen- and estrogen-responsive bioassays, members of a panel of human cell line-based highly selective steroid responsive bioassays. <i>Toxicol. Sci.</i> , 83, 136-48.