CALUX®
Highlight

BioDetection Systems

ERbeta CALUX®

The estrogen receptor beta responsive (ERbeta) 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 ERbeta. Following binding of these compounds to the intracellular ERbeta, 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	ERbeta CALUX
Basal cell line	U2OS
Species	human
Tissue	bone
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 ERbeta 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 being assessed with the cytotox CALUX assay.
Sensitivity (LOD/Q)	Typically in 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	Similar to ERalpha, which is the preferred assay in guideline studies.
HTS protocol	BDS; see EURL-ECVAM DB-ALM Protocol n° 197 : Automated CALUX reporter gene assay procedure
Key reference	Van der Burg, B., Van der Linden, S.C., Man, H.Y., Winter, R., Jonker, L., Van Vugt-Lussenburg, B., Brouwer, A. (2013) A panel of quantitative CALUX® reporter gene assays for reliable high throughput toxicity screening of chemicals and complex mixtures. In "High throughput screening methods in toxicity testing" (P. Steinberg, ed). John Wiley and Sons, Inc. New York. ISBN 9781118065631 pp. 519-532