

cytotox CALUX®

The cytotoxicity responsive CALUX (cytotox CALUX) consists of the human osteosarcoma cell line U2OS, incorporating the firefly luciferase gene coupled to a constitutively active promoter. This leads to a level of luciferase production, and after addition of the appropriate substrate for luciferase, light. Cell toxic compounds repress this signal dose-dependently. This repression can be used as a measure of cytotoxicity, but also other non-specific influences on CALUX assays, and used to exclude non-specific assay interferences. The reference compound used is tributyltin acetate.

Specification	cytotox CALUX
Basal cell line	U2OS
Species	human
Tissue	bone
Positive control	tributyltin acetate
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	Repression of luciferase activity to exclude non-specific assay interferences
Assay interferences	Minimal because of use of highly pathway specific 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 microgram 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. Lower for high potency compound provided in DMSO
Assessment criteria	In house methods, compliant with relevant application/regulations.
SOPs and Guidelines	BDS internal, similar to ER-, and AR CALUX assays
HTS protocol	BDS; see EURL-ECVAM DB-ALM Protocol n° 197 : Automated CALUX reporter gene assay procedure
Key reference	Van der Linden, SC, von Bergh A, Van Vugt-Lussenburg B, Jonker L, Brouwer A, Teunis M, Krul C and Van der Burg B. (2014) Development of a panel of high throughput reporter gene assays to detect genotoxicity and oxidative stress, Mutation Res.760,23-32.