CALUX®
Highlight

BioDetection Systems

P53liver CALUX®

The p53 responsive CALUX consists of the human hepatoma cell line HepG2, incorporating the firefly luciferase gene coupled to p53 responsive elements (p53 REs) as a reporter gene for the presence of p53 pathway acivating ligands. Following activation by these compounds of the p53 pathway, p53 binds to p53 REs. This will lead to expression of proteins that are under normal circumstances associated to p53 REs -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 pathway activation, which is benchmarked against the relevant reference compound, the pathway agonist actinomycin D and expressed as toxic equivalents (TEQs), or bioanalytical equivalents (BEQs).

Specification	P53liver CALUX
Basal cell line	HepG2
Species	human
Tissue	liver
Positive control	actinomycin D
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	Activation of the p53 pathway 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 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 high 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. 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	Budin C, Man HY, Al-Ayoubi C, Puel S, van Vugt-Lussenburg BMA, Brouwer A, Oswald IP, van der Burg B, Soler L. Versicolorin A enhances the genotoxicity of Aflatoxin B1 in human liver cells by inducing the transactivation of the Ah-Receptor. Food Chem Toxicol. 2021 May 10:112258.