CALUX®
Highlight

BDS
BioDetection Systems

DRhuman CALUX®

The human cell-based dioxin responsive (DR) CALUX consists of the human hepatoma cell line HepG2, incorporating the firefly luciferase gene coupled to dioxin responsive elements (DREs) as a reporter gene for the presence of dioxins (PCDDs) and dioxin-like compounds, like furans (PCDFs) and dioxin-like PCBs (dl-PCBs). Following binding of these compounds to the intracellular aryl-hydrocarbon receptor (AhR), the ligand-receptor complex binds the DRE. This will lead to expression of proteins that are under normal circumstances associated to DRE-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 2,3,7,8-TCDD, and expressed as toxic equivalents (TEQs), or bioanalytical equivalents (BEQs). While it is in general less sensitive than the rat cell-based lines, it can be applied in cases where species-specific differences in ligand responses could occur.

Specification	DRhuman CALUX
Basal cell line	HepG2
Species	human
Tissue	liver
Positive control	2,3,7,8-TCDD
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 AhR, through selective sulfuric acid work-up method typically by very stable dioxin-like compounds 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. In addition, for dioxin TEQ assessment in mixtures the sample is cleaned up by a sulfuric acid treatment and afterwards with an additional step to separate dl-PCBs from PCDD/Fs. Cytotoxicity is checked to exclude false-negatives.
Sensitivity (LOD/Q)	Typically in the 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. 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 EURL-ECVAM method DB-ALM Protocol n° 197: Automated CALUX reporter gene assay procedure.
HTS protocol	BDS; see EURL-ECVAM DB-ALM Protocol n° 197 : Automated CALUX reporter gene assay procedure
Key reference	Budin C, Talia C, Besselink H, Van Vugt-Lussenburg B, Swart K, Jonker L, Middelhof I, Brouwer A, Fowler P, Van der Burg B (2021) Assessment of the effect of maternal smoking on placental and foetal hepatic AhR activity using a CALUX reporter gene assay with improved sensitivity, the DRhp CALUX. Thesis chapter, Vrije Universiteit Amsterdam.