CALUX®
Highlight

BioDetection Systems

DRhp CALUX®

The high performance (hp) variant of the dioxin responsive (DR) CALUX consists of the rat hepatoma cell line H4IIE, 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). Being even more sensitive that the standard DR CALUX line, the hp line has been specifically designed to analyse samples of very small size, like in epidemiological studies.

Specification	DRhp CALUX
Basal cell line	H4IIE
Species	rat
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 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 EC COMMISSION REGULATION (EU) No 644/2017, COMMISSION REGULATION (EU) No 771/2017, NL-SPECIE-07 (Rijkswaterstaat, the Netherlands), EPA-4435 (USA), JIS guidelines 463 (Japan), Veileder for risikovurdering av forunenset sediment (TA-2085/2005) (Norway) 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.