CALUX®
Highlight

BDS
BioDetection Systems

PAH CALUX®

The polyhalogenated hydrocarbon (PAH)-responsive PAH 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 carcinogenic PAHs. 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 benzo(a)pyrene (BaP), and expressed as toxic equivalents (TEQs), or bioanalytical equivalents (BEQs). Through the use of a specific reporter gene construct, selective workup and bioanalysis protocols PAH selectivity is obtained.

Specification	PAH CALUX
Basal cell line	H4IIE
Species	rat
Tissue	liver
Positive control	benzo-a-pyrene
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	6hr (incubation time)
Specificity	Binding to the AhR, through specific work-up method and short incubation time typically by PAHs only
Assay interferences	Minimal because of use of highly pathway specific construct, selective workup, and extensive QA/QC. Cytotoxicity is checked to exclude false-negatives.
Sensitivity (LOD/Q)	Typically in ng 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	Pieterse B, Felzel E, Winter R, van der Burg B, Brouwer A (2013) PAH-CALUX, an optimized bioassay for carcinogenic hazard identification of polycyclic aromatic hydrocarbons (PAHs) as individual compounds and in complex mixtures. Environ Sci Technol, 47, 11651-11659.