CALUX®
Highlight

BioDetection Systems

PPARalpha CALUX®

The peroxisome proliferator receptor alpha responsive (PPARalpha) CALUX consists of the human osteosarcoma cell line U2OS, incorporating the human PPARalpha and the firefly luciferase gene coupled to PPAR responsive elements (PPREs) as a reporter gene for the presence of PPAR ligands. Following binding of these compounds to the intracellular PPARalpha, the ligand-receptor complex binds the PPREs. This will lead to expression of proteins that are under normal circumstances associated to PPRE-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 GW7674, and expressed as toxic equivalents (TEQs), or bioanalytical equivalents (BEQs).

Specification	PPARalpha CALUX
Basal cell line	U2OS
Species	human
Tissue	bone
Positive control	GW7674
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 PPARalpha. 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 specific receptor 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. Much 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	Pham Ngoc L, Man HY, Besselink H, Dang Thi Cam H, Abraham Brouwer A, and Van der Burg, B. (2019) Identification of PPAR-activating compounds in herbal and edible plants and fungi from Vietnam using a comprehensive panel of PPAR reporter gene assays. Industrial Crops and Products 129, 195-200.