CALUX®
Highlight

BioDetection Systems

Nrf2 CALUX®

The Nrf2 responsive CALUX consists of the human osteosarcoma cell line U2OS, incorporating the firefly luciferase gene coupled to Nrf2 responsive elements (Nrf2 REs) as a reporter gene for the presence of Nrf2 pathway acivating ligands. Following activation by these compounds of the Nrf2 pathway, Nrf2 binds to Nrf2 REs. This will lead to expression of proteins that are under normal circumstances associated to Nrf2 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 curcumin, and expressed as toxic equivalents (TEQs), or bioanalytical equivalents (BEQs).

Specification	Nrf2 CALUX
Basal cell line	U2OS
Species	human
Tissue	bone
Positive control	curcumin
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 Nrf2 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	Van der Linden, SC, von Bergh A, Van Vugt-Lussenburg B, Jonker L, Brouwer A, Teunis M, Krul C and Van der Burg B. (2014) Development of a panel of high throughput reporter gene assays to detect genotoxicity and oxidative stress, Mutation Res.760,23-32.