

## HIF1alpha CALUX®

The HIF1alpha responsive CALUX consists of the human osteosarcoma cell line U2OS, incorporating the firefly luciferase gene coupled to HIF1alpha responsive elements (HIF1alpha REs) as a reporter gene for the presence of HIF1alpha pathway activating ligands. Following activation by these compounds of the HIF1alpha pathway, HIF1alpha binds to HIF1alpha REs. This will lead to expression of proteins that are under normal circumstances associated to HIF1alpha 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 cobaltous(II)chloride and expressed as toxic equivalents (TEQs), or bioanalytical equivalents (BEQs).

Specification	HIF1alpha CALUX
Basal cell line	U2OS
Species	human
Tissue	bone
Positive control	cobaltous(II)chloride
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 HIF1alpha 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 be 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	Piersma AH, Schulpen SHW, Uibel F, Van Vugt-Lussenburg, B, Bosgra S, Hermsen SAB, Roelofs MJE, Man, H., Jonker, L., Van der Linden, S, Van Duursen MBM, Wolterbeek APM, Schwarz M, Kroese ED, Van der Burg B. (2013) Evaluation of an alternative in vitro test battery for detecting reproductive toxicants. <i>Reprod. Toxicol.</i> 38,53-64.