

AR CALUX®

The androgen receptor responsive (AR) CALUX consists of the human osteosarcoma cell line U2OS, incorporating the firefly luciferase gene coupled to androgen responsive elements (AREs) as a reporter gene for the presence of androgens and androgen-like compounds, as well as human androgen receptor. Following binding of these compounds to the intracellular AR, the ligand-receptor complex binds the AREs. This will lead to expression of proteins that are under normal circumstances associated to ARE-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, dihydrotestosterone (DHT), and expressed as toxic equivalents (TEQs), or bioanalytical equivalents (BEQs).

Specification	AR CALUX
Basal cell line	U2OS
Species	human
Tissue	bone
Positive control	dihydrotestosterone
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 AR 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 specific receptor 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 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, OECD TG458, and relevant protocols similar to ER guidelines
HTS protocol	BDS; see EURL-ECVAM DB-ALM Protocol n° 197 : Automated CALUX reporter gene assay procedure
Key reference	Sonneveld, E., Jansen, H.J., Riteco, J.A.C., Brouwer, A., Van der Burg, B. (2005) Development of androgen- and estrogen-responsive bioassays, members of a panel of human cell line-based highly selective steroid responsive bioassays. <i>Toxicol. Sci.</i> , 83, 136-48.