

## TRbeta CALUX®

The thyroid receptor beta responsive (TRbeta) CALUX consists of the human osteosarcoma cell line U2OS, incorporating the firefly luciferase gene coupled to thyroid responsive elements (TREs) as a reporter gene for the presence of thyroid hormones and thyroid hormone-like compounds, as well as human thyroid receptor beta. Following binding of these compounds to the intracellular TRbeta, the ligand-receptor complex binds the TREs. This will lead to expression of proteins that are under normal circumstances associated to TRE-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, triiodothyronine (T3), and expressed as toxic equivalents (TEQs), or bioanalytical equivalents (BEQs).

Specification	TRbeta CALUX
Basal cell line	U2OS
Species	human
Tissue	bone
Positive control	triiodothyronine
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 TR. 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 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	Collet B, Simon E, Van der Linden S, El Abdellaoui N, Naderman M, Man HY, Middelhof I, Van der Burg B, Besselink H, Brouwer A. (2019) Validation of a panel of in vitro methods for assessing thyroid receptor $\beta$ and transthyretin transporter disrupting activities. <i>Reproductive Toxicol.</i> , 18, 30554-30559