CALUX®
Highlight

BioDetection Systems

# PXR CALUX®

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

| Specification | PXR CALUX |
|---|---|
| Basal cell line | U2OS |
| Species | human |
| Tissue | bone |
| Positive control | nicardipine |
| 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 PXR 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 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 | 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. Reprod. Toxicol. 38,53-64. |