

# Research Brief 204: CALUX Generation 3 – Enhanced Sensitivity for Low Volume/Low Concentration Samples

Release Date: 12/07/2011



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## Background

Dr. Michael Denison's SRP-funded research at the University of California-Davis SRP led to the development and validation of a series of mechanistically-based bioassays for rapid and inexpensive detection of dioxin-like HAHs. To develop the Chemically Activated Luciferase Expression bioassay (CALUX), the researchers placed a firefly luciferase gene under the control of the AhR and inserted it into a mouse cell line. Dioxin-like HAHs in a sample bind to the AhR and activate the receptor. The AhR-contaminant complex then travels into the nucleus of the cell and binds to specific sequences in the DNA (dioxin responsive elements [DREs]). This induces expression of firefly luciferase, which results in dose-dependent production of light that is easily measured and provides accurate and reproducible detection and relative quantitation of dioxin-like chemicals in a wide variety of biological, environmental, and food/feed samples.

CALUX received regulatory certification as a validated method by the US Environmental Protection Agency ([Method 4435](#)) and has been used world-wide (see [Research Brief 150](#)). However, the sensitivity and responsiveness of the assay are not sufficient for CALUX to be used for analysis of very low volume or very low concentration samples.

## Advances

Dr. Denison's research team worked to increase the responsiveness of the CALUX cell bioassay by continuing their strategy of focusing on the mechanisms by which contaminants affect cellular receptors, signal transduction pathways, or cellular/enzyme functions.

To enhance the CALUX bioassay, the researchers increased the number of DREs/plasmid (there were 4 in first and second generation CALUX assays). They evaluated systems with 4 - 40 DREs/plasmid and determined that sensitivity and response were maximized in systems with 20 DREs. They generated a series of stably transfected third generation (G3) CALUX bioassays using cell lines from four different species and selected mouse hepatoma (Hepa1c1c7) cells as the optimal G3 cell line. *The detection limit and sensitivity of the G3 CALUX were lowered by a factor of 10-100 fold compared to G2.* The minimum detection limit (MDL) for dioxin (TCDD) for the CALUX G3 is 0.01 pM.

The G3 CALUX bioassay allowed for analysis of dioxins, furans, and dioxin-like PCBs in samples from the Flemish Environment and Health Study (FLEHS II). Although screening by gas chromatography-high resolution mass spectrometry (GC-HRMS) was not possible because of the low volume of the samples, Dr. Denison and his collaborators were able to detect dioxins, furans, and dioxin-like PCBs in serum samples of adolescents and adults. The results will be used as a reference value and will allow the researchers to determine the pollution pressure in specific hot spots and to follow up the concentration levels in adolescents over a period of time. This study included robust quality control/quality assurance protocols. Validation studies showed that the relative standard deviation for the repeatability and within-lab reproducibility for the QC standard were lower than the in-house quality control criteria limits.

## Significance

Amplification of aspects of the molecular mechanism of dioxin action has allowed the Denison research group to improve the sensitivity of detection of the CALUX cell bioassay system for detection and relative quantitation of dioxin and related chemicals by 10- to 100-fold.

The increased responsiveness and lower MDL of the G3 CALUX bioassay provide new opportunities to expand both the format of the bioassay and its applications. The more responsive analysis requires fewer cells per well and has allowed the assay to be carried out in 384- and 1536-well microplate formats, further reducing sample and reagent costs and increasing sample

**Halogenated aromatic hydrocarbons (HAHs)** are chemicals that contain one or more atoms of a halogen (chloride, fluoride, bromide, or iodide) and a benzene ring. "Dioxin-like HAHs" (dioxins, furans, and polychlorinated biphenyls [PCBs]) are persistent, ubiquitous and highly lipophilic environmental contaminants that have been detected in soil, sediment, water, air, and diverse biological matrices. Exposure to dioxin-like HAHs can result in a broad spectrum of species- and tissue-specific toxic and biological effects (e.g., birth defects, immunotoxicity, tumor production) that are primarily mediated by the aryl hydrocarbon receptor (AhR).

throughput. The increased sensitivity of the G3 bioassay will facilitate screening analysis of samples containing low levels of dioxin-like chemicals and those where the sample matrices for analysis are very limiting (e.g., large epidemiology studies).

The new CALUX bioassay has been provided to governmental, academic, and commercial laboratories in six countries and is currently being evaluated for adoption as a validated screening method to detect the presence of these toxic chemicals in food and feed. The G3 bioassay system is currently being evaluated by the European Union (EU) Reference Laboratory for Dioxins and PCBs in Feed and Food in Germany for consideration as the dioxin screening method of choice for governmental regulatory laboratories in the EU member states. The UC Davis Innovation Access technology-transfer office has made this technology available for research and commercial licensing.

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**To learn more about this research, please refer to the following sources:**

He, G., T. Tsutsumi, B. Zhao, D.S. Baston, J.Zhao, S. Heath-Pagliuso, and M S. Denison. 2011. Third-Generation Ah Receptor-Responsive Luciferase Reporter Plasmids: Amplification of Dioxin-Responsive Elements Dramatically Increases CALUX Bioassay Sensitivity and Responsiveness. *Toxicological Sciences* 123(2), 511–522.  
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[doi:10.1016/j.talanta.2011.07.103](https://doi.org/10.1016/j.talanta.2011.07.103)

