BioDetection Systems

CALUX bioassays

BDS's CALUX Catalogue

Feed/Food Safety

Water Safety

Environmental Safety

Pharmaceutical/Chemical Safety

Consumer Products Safety

Clinical Testing

Cell biology Products

Reference Materials

Other Products

The Reportergene Bioassay Experts

www.bds.nl

Overview

Your Partner for research & development!

Overview

Your Partner for service analysis !

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e) Pig Meat.....

f) Fish BRM-08.....

8 OTHER PRODUCTS (ON REQUEST VIA WEBPAGE)

Standards.....

About BioDetection Systems (BDS)

Over 30 years ago the team of Prof. Brouwer started its development of cell- and effect-based bioanalysis tools in the Wageningen. Wageningen is not only home to one of the most prestigious universities in Netherlands but it is also popular for research related businesses. Afterwards the team of Prof. Brouwer moved to the Free University of Amsterdam. Amsterdam provides the ideal scientific environment for international business and serves as well-known & easy to reach city. With rapidly increasing international projects, in 2001 Prof. Brouwer founded BioDetection Systems as Spin-off Company of his R&D activities at the Free University of Amsterdam. Since 2005 BDS is in the prestigious Science Park of Amsterdam. In this location an ideal R&D environmental exists surrounded from many other bio-based companies.

Since 1995 the team of Prof. Brouwer is organizing trainings and workshops for effect-based analysis for all kinds of chemicals and cocktails of dose.

Since 2005, our dioxin and hormone service analysis laboratory is ISO 17025 (L401) accredited according to current national or international standards.

Dear Customer,

At BDS we are proud to work together successfully with scientists worldwide, providing them with high quality products and experienced technical customer support. With over 50 CALUX in vitro reportergene assays we have the tools to meet the needs of your research project!

Under our **CALUX** brand we offer a broad range of cell- and effect-based bioanalysis tools as well as optimized cell culture media, standards, and reference materials. Scientists worldwide use CALUX tests in basic and applied bio-based research to obtain better results for the risk assessment of chemicals, pharmaceuticals & their cocktails. The reliability of our cells provides the basis for being able to offer you the high-quality standards you need.

For more in-depth analysis of our bioanalysis tools, why not to look also at our webpage with many different applications (see at library) and the latest news? BDS offers also online our quality kits and reagents for all our cell-based analysis tools (see at “Request to BDS/Order format”).

To make the establishment of the CALUX technologies in your laboratory as easy as possible we also offer all kinds of trainings related to our services and products. Our courses with state-of-the-art insight and up to date trends in the Bioanalysis Sciences in a professional and hands-on setting. You can choose from a growing collection of course topics in English as well as in German and Nederland’s.

We are looking forward to working with you and providing you with our support!

Scientists supporting Scientists – guaranteed.

Your BDS Team

Discover our Knowledge Base!

Information at your fingertips

As scientists, we know that research is not an easy task. Scientists in the 21st century need information fast, accurate, reliable and at any time, day, or night.

To assist you with this as good as possible, BDS has created our online library. At the touch of a button, you can now find a wealth of product- and application related and technical information to support your research needs.

We appreciate your feedback.

We are continually improving our products and services to make them fit your needs. Your feedback, ideas and suggestions therefore are valued and important to us.

Therefore, we are always looking forward to your feedback! We thank you in advance for providing us with your ideas and suggestions.

Of course, our customer support team is also happy to receive your feedback via email, fax, or telephone.

To find your personal contact, please refer to the contact section on our website.

Sections of the Knowledge Base include:

Technical Library
Application Notes
Reference Literatures

You can find detailed answers to many questions about our products in the section “Library”. Type in a keyword and the Knowledge Base will show you the associated technical questions and answers within seconds.

In the section “Application Notes” you will find documents which supplement the information in our Product Manuals
(e. g. detailed protocols and procedures).

If you are interested in publications by our customers, the section “Reference Literature” offers you access to literary citations of scientists that use our CALUX products and services.

Overview about our different applications (page 1)

	Dioxins	Hormone	PAH	PFAS	Geno toxicity	General toxicity
Anabolic steroids		x	x		x	x
Authenticity (e.g. horse meat)						Via QPCR
Blood/ Human tissues/mother milk	x	x	x	x	x	x
Chemicals & Complex mixtures	x	x	x	x	x	x
DNA laboratory						Via QPCR and ion torrent
Emissions/Dust/ Ashes	x	x	x	x	x	x
Feed/Food	x	x	x	x	x	x
Food Contact Materials		x	x	x	x	x
Pharmaceuticals	x	x	x	x	x	x

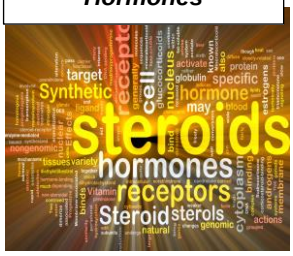
Overview about our different applications (page 2)

	Dioxins	Hormone	PAH	PFAS	Geno toxicity	General toxicity
Veterinary residue drugs	x	x	x	x	x	x
Sport Doping: Blood/Urine	x	x	x	x	x	x
Hazardous Waste	x	x	x	x	x	x
Soil/Sediment	x	x	x	x	x	x
Water	x	x	x	x	x	x
Wildlife	x	x	x	x	x	x
& others on your demand	x	x	x	x	x	x

..by Non Animal Testing

Overview about our different applications (visual)

Hormones



Non-animal testing

Blood/mother milk

Mixtures

DNA Laboratory

Gas/Ashes

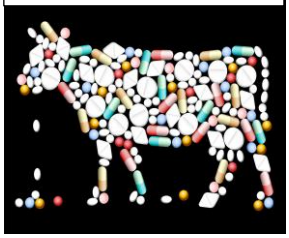
Feed

Food

Consumer Products

Pharmaceutials

Veterinary Drugs

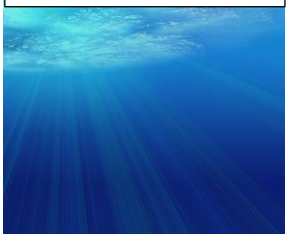


Anabolic Steroids

Hazardous Waste

Environment

Water



Wildlife

Overview about our compound activity profiles.

Bioactive Compounds	HTPS CALUX	Pathway
C- and N-Dioxins PXDD/Fs, dl-PXBs (X= Cl, Br, F, methyl)	DR CALUX	Dioxin receptor
Carcinogenic PAHs (such as Benzo(a)pyrene)	PAH CALUX	Dioxin receptor
Estrogens, EDCs, Bisphenol A, Phthalates, Pesticides, Pharmaceuticals, cosmetics	ER CALUX	Estrogen receptor mix
Androgens, EDCs, Bisphenol A, Pesticides, Pharmaceuticals	AR CALUX	Androgen receptor
Progestins, EDCs, Anti-baby pill, Pesticides, Pharmaceuticals	PR CALUX	Progesterone receptor
Glucocorticoids, EDCs, Asthma spray, Immune-suppressive agents	GR CALUX	Glucocorticoid receptor
Thyroid hormones, EDCs, Brominated flame retardants	TR, TTR-TR PFAS CALUX	Thyroid receptor
Retinoids, Pesticides, Pharmaceuticals	RAR CALUX	Retinoic acid receptor
Obesogens, fluorinated compounds PFAAs, Anti-diabetic pharmaceuticals	PPARgamma CALUX	Peroxisome proliferatorϒ1 receptor
Obesogens, fluorinated compounds PFAAs, Anti-diabetic pharmaceuticals	PPARalpha CALUX	Peroxisome proliferatorα receptor
Pro-inflammatory cytokines	NFkappaB CALUX	NFkappaB activation
Cytotoxic/static agents, Genotoxic compounds like PAHs, Pharmaceuticals, dyes	p21 CALUX	p21 activation
Cytotoxic/static agents, Genotoxic compounds like PAHs, Pharmaceuticals, dyes	p53 +/- S9 CALUX	p53 transcriptional activity
Electrophiles, oxidative stress, heavy metals	Nrf2 CALUX	Nrf2 transcriptional activity
β-Catenin/ involved in development and carcinogenesis	TCF	TCF transcriptional activity
Carcinogens, UV	AP1 CALUX	AP1 transcriptional activity
Hypoxia-mediated angiogenesis	HIF1alpha CALUX	HIF1α transcriptional activity
Endoplasmatic reticulum stressors (ESRE)	ESRE CALUX	XBP1 transcriptional activity
Cytotoxic agents, Non-specific luciferase modulators	Cytox CALUX	Constitutive transcriptional activity

Why effect- and cell-based bioanalysis?

Robotics

Originally, CALUX reporter gene assays have been performed manually in 96-wells plates. In recent years, however, liquid handling robots that can accurately perform routine experiments have entered the market.

At present, most steps of the CALUX assay have been automated, and more recently, the procedure has also been miniaturised in 384-wells format. This automation and miniaturisation have significantly increased both throughput and accuracy.

Furthermore, in this miniaturised set-up, only one-third of the original sample volume is required, which is especially relevant when only little sample is available, such as in clinical or epidemiological studies.

BDS offers the favorably priced automated 384-wells set-up if demanded by you.

It is also possible to select multiple sets of CALUX® assays.

DR CALUX (Catalog No. 001)

The Dioxin Responsive (DR) CALUX® comprise rat hepatoma cell lines (H4IIE), incorporating the firefly luciferase gene coupled to Dioxin Responsive Elements (DREs) as a reporter gene for the presence of dioxins (PCDDs) and dioxin-like compounds (e.g., furans (PCDFs) and dioxin-like PCBs (dl-PCBs)). Following binding of dioxins and/or dioxin-like compounds to the cytosolic Aryl-hydrocarbon receptor (AhR), the ligand-receptor complex binds the DRE. Cells that are exposed to dioxins or dioxin-like compounds not only express proteins that are under normal circumstances associated to DRE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds (2, 3, 7, 8-TCDD). DR CALUX bioassays report total 2,3,7,8-TCDD TEQs for environmental matrices and total BEQs for food/feed matrices.

- **Endpoint (unit):** ng 2,3,7,8-TCDD equivalents/kg sample processed.
- **Test duration:** 24h incubation time.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: EC COMMISSION REGULATION (EU) No 644/2017, COMMISSION REGULATION (EU) No 771/2017, NL-SPECIE-07 (Rijkswaterstaat, the Netherlands), EPA-4435 (USA), JIS guidelines 463 (Japan), Veileder for risikovurdering av forunenset sediment (TA-2085/2005) (Norway) and ECVAM method DB-ALM Protocol n° 197.: Automated CALUX reporter gene assay procedure.
- **Positive control used**: 2,3,7,8-TCDD.
- **Matrices (food/feed, blood, mother milk, sediment, water etc.) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Rat liver cell line H4IIE.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ). The bioassays' LOQ is 1 pg 2, 3,7,8-TCDD equivalents per amount of material processed. For example, 5 grams of dried soil/sediment/feed or 1 liter of water is processed resulting in a LOQ of 0.2 ng 2,3,7,8-TCDD equivalents per gram of soil/sediment/feed or 1 ng 2,3,7,8-TCDD equivalents per liter of water respectively.

- **Current use as screening tool (regular monitoring as well as other purposes):**
  Food/Feed (international in many countries)
  Sediment/dredged material/sludge-monitoring (RWS-RIKZ)
  Water
  Blood/human milk etc.

- **Assessment criteria:** For chemical assessment of dioxins and planar PCBs, assessment criteria are available for food and feed (EU regulation), but bio-based screening approach fully accepted. For assessment of sediments/dredged materials/sludge and bio waste, the Dutch guidelines are 50 ng TEQ/kg dry weight. The Norwegian guidelines are 25 ng TEQ/kg dry weights, and the Japanese guidelines are 150 ng TEQ/kg dry weight by using the DR CALUX technology.

- **Specificity:** Ah receptor active compounds, e.g., polyhalogenated dioxins/furans (X = Cl, Br, F, J), dioxin like PCBs/PBBs, and if using other pre-treatment of samples also PAHs (see PAH CALUX).

- **Sensitivity (LOD/Q):** In case of feed/food in general 1/5th of the EC regulated levels. For example, 5 grams of dried soil/sediment/feed or 1 liter of water is processed resulting in a LOQ of 0.2 ng 2,3,7,8-TCDD equivalents per gram of soil/sediment or 1 ng 2,3,7,8-TCDD equivalents per liter of water, respectively.

- **Variability (e.g., CV for single substance tests):** <20%

- **Influence by cytotoxicity/risk of false positives/negatives:** As the sample is cleaned up by a sulfuric acid treatment and afterwards with an additional step to separate dl-PCBs from PCDD/Fs, cytotoxicity is rarely occurring. In case of false positive/false negative guided levels must be established to compare it with (e.g., EC/644/2017 below 5% false compliant rate accepted).

- **Complexity/learning period:** 2 weeks of training at BDS

- **Costs:** Costs are generally depending on matrix studied.

- **Commercial availability:** via BDS

- **International relevance:** Standardized test for feed/food according to many international regulations (such as EC/644/2017 or EC/709/2014). Standardized test for sediments to several international standards (such as Dutch Specie, JIS or US-EPA). For water analysis it is proposed according to EC/105 (2008) guideline.

- **Publications:** please see for more info at www.bds.nl under literature.

Typical applications








PAH CALUX (Catalog No. 002)

The PAH Responsive (PAH) CALUX® comprise rat hepatoma cell lines (H4IIE), incorporating the firefly luciferase gene coupled to Dioxin Responsive Elements (DREs) as a reporter gene for the presence of poly aromatic hydrocarbons (PAHs). Following binding of PAHs to the cytosolic Arylhydrocarbon receptor (AhR), the ligand-receptor complex binds the DRE. Cells that are exposed to PAHs not only express proteins that are under normal circumstances associated to DRE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds benzo(a)pyrene (B(a)P). PAH CALUX bioassays report total B(a)P equivalents for environmental and food/feed matrices.

- **Endpoint (unit):** pg B(a)P equivalents/g sample processed.
- **Test duration:** 6h
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: ECVAM method DB-ALM Protocol n° 197.: Automated CALUX reporter gene assay procedure.
-
- **Positive control used**: Benzo(a)pyrene.
- **Matrices (sediment, water, tissue etc) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Rat liver cell line H4IIE.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ). The bioassays' LOQ is 0.45 ng B(a)P equivalents per amount of material processed. For example, 5 grams of dried soil/sediment or 1 liter of water is processed resulting in a LOQ of 0.09 ng B(a)P equivalents per gram of soil/sediment or 0.45 ng B(a)P equivalents per liter of water, respectively.
- **Assessment criteria:** e.g., applied in one official EC project called FaceIt for oil spills.
- **Specificity:** Ah receptor active compounds, e.g., benzo(a)pyrene like compounds is dominating the BaP-equivalents here reported. Especially the higher aromatic PAHs having high activity in the PAH CALUX.
- **Sensitivity (LOD/Q):** please ask for more info for your specific matrix.

- **Variability (e.g., CV for single substance tests) if known:** <20%
- **Influence by cytotoxicity/risk of false positives/negatives:** depending on the clean-up systems and separation technology.
- **Complexity/learning period:** 1 week of training.
- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** Commercial performers available.

- **International relevance:** several PAHs (including benzo(a)pyrene, used as positive control in this test) are already considered priority substances (2008/105/EC), the PAHs that are suspected to induce a response in this bioassay can also include a high level of non-parent PAH structures. To use the EQS for benzo(a)pyrene to evaluate the results is therefore recommended and the assay is very valuable on screening level to identify water bodies at risk of exposure to many relevant PAHs that are normally not analysed chemically.
- **Publications:** please see for more info at www.bds.nl under literature.

Typical applications





ERα CALUX (agonistic/antagonistic) (Catalogue No. 003)

The ERα Responsive (ERα) CALUX® comprise a human bone cell line (U2OS), incorporating the firefly luciferase gene coupled to Estrogen Responsive Elements (EREs) as a reporter gene for the presence of estrogens and/or estrogen-like compounds. Following binding of estrogens or estrogen-like compounds to the cytosolic estrogen receptor, the ligand-receptor complex binds the ERE. Cells that are exposed to estrogens and/or estrogen-like compounds not only express proteins that are under normal circumstances associated to ERE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds 17β-estradiol. ERα CALUX bioassays report total 17β-estradiol equivalents for environmental matrices.

- **Endpoint (unit):** pg 17β-estradiol equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: ISO 19040-3 (water/waste water); OECD TG455-like; Dutch Rijkswaterstaat RIKZ-Specie-08 guideline; Australian Water Commission; EPA California, OECD VMG-NA, and ECVAM method DB-ALM Protocol n° 197.: Automated CALUX reporter gene assay procedure.
- **Positive control used**: 17β-estradiol/tamoxifen (anti).
- **Matrices (sediment, water, tissue etc.) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human bone cell line U2OS.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ). The bioassays' LOQ is 35 pg 17β-estradiol equivalents per amount of material processed. For example, 5 grams of dried soil/sediment or 1 liter of water is processed resulting in a LOQ of 7 pg 17β-estradiol equivalents per gram of soil/sediment or 35 pg 17β-estradiol equivalents per liter of water, respectively.

- **Current use on MS level (regular monitoring as well as other purposes):** Rhine/Meuse/Schelde-monitoring (RWS-RIKZ, the Netherlands), National Water Report – Waterlines 48 report (Australia), Oekotoxzentrum-EAWAG (Switzerland).

- **Assessment criteria:** Dutch Rijkswaterstaat RIKZ-Specie-08 guideline; OECD TG455, ISO 19040-3.

- **Specificity:** Binding and activation of the Estrogen receptor (alpha and beta for original ER CALUX and only alpha for ERalpha CALUX).

- **Sensitivity (LOD/Q):** Original ER CALUX: 0.1 ng EEQ/l water.

- **Variability (e.g., CV for single substance tests) if known:** <20%.

- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as which kinds of water matrixes.

- **Complexity/learning period:** 1 week of training.

- **Costs:** Costs are generally depending on matrix studied.

- **Commercial availability:** BDS.

- **Water framework Directives (WFD) relevance:** E1, EE2 and E2 suggested to be included in 2008/105/EC (EC watchlist already included).

  Effect-based trigger values used in CH and NL between 0,4 – 0,5 ng EEQ/l water (van der Oost et al 2017).

- **Publications:** please see for more info at www.bds.nl under literature

ERß CALUX (agonistic/antagonistic) (Catalogue No. 004)

The ERß Responsive (ERα) CALUX® comprise a human bone marrow cell lines (U2OS), incorporating the firefly luciferase gene coupled to Estrogen Responsive Elements (EREs) as a reporter gene for the presence of estrogens and/or estrogen-like compounds. Following binding of estrogens or estrogen-like compounds to the cytosolic estrogen receptor, the ligand-receptor complex binds the ERE. Cells that are exposed to estrogens and/or estrogen-like compounds not only express proteins that are under normal circumstances associated to ERE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds 17β-estradiol. ERα CALUX bioassays report total 17β-estradiol equivalents for environmental matrices.

- **Endpoint (unit):** pg 17β-estradiol equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: follows similar QA/QC as ERα CALUX.
- **Positive control used**: 17β-estradiol.
- **Matrices (sediment, water, tissue etc.) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human bone marrow cell line U2OS.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ). The bioassays' LOQ is 35 pg 17β-estradiol equivalents per amount of material processed. For example, 5 grams of dried soil/sediment or 1 liter of water is processed resulting in a LOQ of 7 pg 17β-estradiol equivalents per gram of soil/sediment or 35 pg 17β-estradiol equivalents per liter of water, respectively.
- **Current use on MS level (regular monitoring as well as other purposes):** Rhine/Meuse/Schelde-monitoring (RWS-RIKZ, the Netherlands), National Water Report – Waterlines 48 report (Australia), Oekotoxzentrum-EAWAG (Switzerland).
- **Assessment criteria:** Dutch Rijkswaterstaat RIKZ-Specie-08 guideline.

- **Specificity:** Binding to the Estrogen receptor (alpha and beta for original ER CALUX and only alpha for ERalpha CALUX).

- **Sensitivity (LOD/Q):** Original ER CALUX: 0.1 ng EEQ/l water.

- **Variability (e.g., CV for single substance tests) if known:** <20%

- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as which kinds of water matrixes.

- **Complexity/learning period:** 1 week of training.

- **Costs:** Costs are generally depending on matrix studied.

- **Commercial availability:** BDS.

- **Water framework Directives (WFD) relevance:** E1, EE2 and E2 suggested to be included in 2008/105/EC (watchlist already).

- **Publications:** please see for more info at www.bds.nl under literature**.**

ERαß CALUX (agonistic/antagonistic) (Catalogue No. 005)

The ERα Responsive (ER) CALUX® comprise a human cell lines (T47D), incorporating the firefly luciferase gene coupled to Estrogen Responsive Elements (EREs) as a reporter gene for the presence of estrogens and/or estrogen-like compounds. Following binding of estrogens or estrogen-like compounds to the cytosolic estrogen receptor, the ligand-receptor complex binds the ERE. Cells that are exposed to estrogens and/or estrogen-like compounds not only express proteins that are under normal circumstances associated to ERE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds 17β-estradiol. ERα CALUX bioassays report total 17β-estradiol equivalents for environmental matrices.

- **Endpoint (unit):** pg 17β-estradiol equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: Dutch Rijkswaterstaat RIKZ-Specie-08 guideline; ISO 19040-3.
- **Positive control used**: 17β-estradiol.
- **Matrices (sediment, water, tissue etc.) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human breast cancer cell line T47D.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ). The bioassays' LOQ is 35 pg 17β-estradiol equivalents per amount of material processed. For example, 5 grams of dried soil/sediment or 1 liter of water is processed resulting in a LOQ of 7 pg 17β-estradiol equivalents per gram of soil/sediment or 35 pg 17β-estradiol equivalents per liter of water, respectively.
- **Current use on MS level (regular monitoring as well as other purposes):** Rhine/Meuse/Schelde-monitoring (RWS-RIKZ, the Netherlands), National Water Report – Waterlines 48 report (Australia), Oekotoxzentrum-EAWAG (Switzerland).
- **Assessment criteria:** Dutch Rijkswaterstaat RIKZ-Specie-08 guideline.
- **Specificity:** Binding to the Estrogen receptor (alpha and beta for original ER CALUX and only alpha for ERalpha CALUX).

- **Sensitivity (LOD/Q):** Original ER CALUX: 0.1 ng EEQ/l water.
- **Variability (e.g. CV for single substance tests) if known:** <20%.
- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as which kinds of water matrixes.
- **Complexity/learning period:** 1 week of training.
- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** BDS.
- **Water framework Directives (WFD) relevance:** E1, EE2 and E2 suggested to be included in 2008/105/EC (watchlist already).
- **Publications:** please see for more info at www.bds.nl under literature.

Typical applications for all ER CALUX tests



AR CALUX (agonistic/antagonistic) (Catalogue No. 006)

The AR Responsive (AR) CALUX® comprise a human bone marrow cell lines (U2OS), incorporating the firefly luciferase gene coupled to Androgenic Responsive Elements (AREs) as a reporter gene for the presence of androgens and/or androgen-like compounds (such as Bisphenol A). Following binding of androgens or androgen-like compounds to the cytosolic estrogen receptor, the ligand-receptor complex binds to the ARE. Cells that are exposed to androgens or androgen-like compounds not only express proteins that are under normal circumstances associated to ARE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds di-hydro-testosterone (DHT). AR CALUX bioassays report total DHT equivalents for environmental matrices.

- **Endpoint (unit):** pg DHT equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: in-house method, OECD TG458 and ECVAM method DB-ALM Protocol n° 197.: Automated CALUX reporter gene assay procedure.
- **Positive control used**: DHT/Flutamide.
- **Matrices (sediment, water, tissue etc.) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human bone marrow cell line.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ). The bioassays' LOQ is ca. 350 pg DHT equivalents per amount of material processed. For example, 5 grams of dried soil/sediment or 1 liter of water is processed resulting in a LOQ of ca. 70 pg DHT equivalents per gram of soil/sediment or 350 pg DHT equivalents per liter of water, respectively.

- **Current use on MS level (regular monitoring as well as other purposes):** Rhine/Meuse/Schelde-monitoring (RWS-RIKZ, the Netherlands), National Water Report – Waterlines 48 report (Australia), Oekotoxzentrum-EAWAG (Switzerland) and many others.

- **Assessment criteria:** typical performance criteria from Dutch Rijkswaterstaat RIKZ-Specie-08 guideline can be used.

- **Specificity:** Binding to the Androgen receptor.

- **Sensitivity (LOD/Q):** Original AR CALUX: ca.1 ng DHT/l water.

- **Variability (e.g., CV for single substance tests) if known:** <20%

- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as which kinds of water matrixes.

- **Complexity/learning period:** 1 week of training.

- **Costs:** Costs are generally depending on matrix studied.

- **Commercial availability:** BDS.

- **Water framework Directives (WFD) relevance:** Bisphenol A (anti-AR) suggested to be included in 2008/105/EC.

  Elevated levels found in Australia, NL, and CH. Published trigger of 25 µg FluEQ/l water (van der Oost et al 2017).

- **Publications:** please see for more info at www.bds.nl under literature.

Typical applications for AR CALUX test



TRβ CALUX (agonistic/antagonistic) (Catalogue No. 007)

The TRβ Responsive (TRβ) CALUX® comprise a human bone marrow cell lines (U2OS), incorporating the firefly luciferase gene coupled to Thyroid Responsive Elements (TREs) as a reporter gene for the presence of thyroid-like compounds. Following binding of thyroid -like compounds to the cytosolic thyroid receptor, the ligand-receptor complex binds the TRE. Cells that are exposed to thyroid-like compounds not only express proteins that are under normal circumstances associated to TRE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds T3. TR CALUX bioassays report total T3 equivalents for environmental matrices.

- **Endpoint (unit):** pg T3 equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol):** in house method, in preparations of OECD WGs and ECVAM method DB-ALM Protocol n° 197.: Automated CALUX reporter gene assay procedure.
- **Positive control used:** T3.
- **Matrices (sediment, water, tissue etc.) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human bone marrow cell line U2OS.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ). The bioassays' LOQ is ca. 3 ng DHT equivalents per amount of material processed. For example, for 1 liter of water is processed resulting in a LOQ of ca. 3 ng T3 equivalents per per liter of water, respectively.
- **Current use on MS level (regular monitoring as well as other purposes):** Rhine/Meuse/Schelde-monitoring (RWS-RIKZ, the Netherlands), National Water Report – Waterlines 48 report (Australia).
- **Assessment criteria:** in house method.
- **Specificity:** Binding to the thyroid receptor.
- **Sensitivity (LOD/Q):** Original TR CALUX: ca.3 ng T3 EQ/l water.
- **Variability (e.g., CV for single substance tests) if known**: <20%.

- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as which kinds of water matrixes.
- **Complexity/learning period**: 1 week of training.
- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** BDS.
- **Water framework Directives (WFD) relevance:** not yet evaluated.
- **Publications:** please see for more info at www.bds.nl under literature.

Typical applications for TR CALUX test




GR CALUX (agonistic/antagonistic) (Catalogue No. 008)

The GR Responsive (GR) CALUX® comprise a human bone marrow cell lines (U2OS), incorporating the firefly luciferase gene coupled to Glucocorticoid Responsive Elements (GREs) as a reporter gene for the presence of glucocorticoid-like compounds. Following binding of glucocorticoid -like compounds to the cytosolic glucocorticoid receptor, the ligand-receptor complex binds the GRE. Cells that are exposed to glucocorticoid -like compounds not only express proteins that are under normal circumstances associated to GRE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds dexamethason. GR CALUX bioassays report total dexamethason equivalents for environmental matrices.

- **Endpoint (unit):** pg dexamethasone equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: ECVAM method DB-ALM Protocol n° 197.: Automated CALUX reporter gene assay procedure.
- **Positive control used**: dexamethasone.
- **Matrices (sediment, water, tissue etc) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human bone marrow cell line U2OS.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ). The bioassays' LOQ is ca. 20 pg dexamethasone equivalents per amount of material processed. For 1 liter of water is processed resulting in a LOQ of ca. 20 pg dexamethasone equivalents per liter of water, respectively.
- **Current use on MS level (regular monitoring as well as other purposes):** Rhine/Meuse/Schelde-monitoring (RWS-RIKZ, the Netherlands), Australia National Water Commission, USA California EPA also focus on this relevant endpoint.
- **Assessment criteria:** in house method.
- **Specificity:** Binding to the glucucorticoid receptor.

- **Sensitivity (LOD/Q):** Original GR CALUX: ca.20 pg dexamethasone EQ/l water.
- **Variability (e.g., CV for single substance tests) if known:** <20%.
- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as which kinds of water matrixes.
- **Complexity/learning period:** 1 week of training.
- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** BDS.
- **International relevance:** Elevated levels found in Australia, Japan, NL, and CH. Published trigger of 100 ng DexEQ/ l water (van der Oost et al 2017).
- **Publications:** please see for more info at www.bds.nl under literature.

Typical applications for GR CALUX test




PR CALUX (agonistic/antagonistic) (Catalogue No. 009)

The PR Responsive (PR) CALUX® comprise a human bone marrow cell lines (U2OS), incorporating the firefly luciferase gene coupled to Progesterone Responsive Elements (PREs) as a reporter gene for the presence of progestin-like compounds. Following binding of progestin-like compounds to the cytosolic progestin receptor, the ligand-receptor complex binds the PRE. Cells that are exposed to progestin-like compounds not only express proteins that are under normal circumstances associated to PRE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds Org 2058. PR CALUX bioassays report total Org 2058 equivalents for environmental matrices.

- **Endpoint (unit):** pg Org 2058 equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: ECVAM method DB-ALM Protocol n° 197.: Automated CALUX reporter gene assay procedure.
- **Positive control used**: Org 2058/ Ru486 (anti-).
- **Matrices (sediment, water, tissue etc) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human bone marrow cell line.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ). The bioassays' LOQ is ca. 20 pg Org 2058 equivalents per amount of material processed. For 1 liter of water is processed resulting in a LOQ of ca. 20 pg Org 2058 equivalents per liter of water, respectively.
- **Current use on MS level (regular monitoring as well as other purposes):** Rhine/Meuse/Schelde-monitoring (RWS-RIKZ, the Netherlands), Australia National Water Commission, USA California EPA also focus on this relevant endpoint.

- **Assessment criteria:** typical performance criteria from Dutch Rijkswaterstaat RIKZ-Specie-08 guideline can be used.
- **Specificity:** Binding to the progestin receptor.
- **Sensitivity (LOD/Q):** Original PR CALUX: ca.20 pg Org 2058 EQ/l water.
- **Variability (e.g., CV for single substance tests) if known:** <20%.
- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as which kinds of water matrixes.
- **Complexity/learning period:** 1 week of training.
- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** BDS.
- **International relevance:** Many compounds of EU-WFD are anti-PR, but not many results are available yet.

- **Publications:** please see for more info at www.bds.nl under literature.

Typical applications for PR CALUX test




PFAS CALUX (Catalogue No. 010)

The PFAS CALUX® comprise a human bone marrow cell lines (U2OS), incorporating the firefly luciferase gene coupled to Thyroid Responsive Elements (TREs) as a reporter gene for the presence of thyroid-like inhibiting compounds. He is based on the TTR-binding assay in combination with the TRβ CALUX bioassay. In the TTR-binding assay, binding competition between a fixed concentration of T4 and dilution series of test items are studied. In the presence of increasing concentrations of PFAS capable of competing with T4 for TTR-binding sites will result in a decreased amount of TTR-bound T4. Following separation of TTR-bound and free T4, the amount of TTR-bound T4 is determined using the TRβ CALUX bioassay. Disruption of T4-TTR binding is benchmarked against the reference compound PFOA (REP = 1).

- **Endpoint (unit):** pg PFOA equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol):** in house method.
- **Positive control used:** PFOA, PFOS or any other PFAS on demand.
- **Matrices (sediment, water, tissue etc.) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human bone marrow cell line U2OS.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ).
- **Current use on MS level (regular monitoring as well as other purposes):** Food, water, soil, blood.
- **Assessment criteria:** in house method.
- **Specificity:** Competition of the PFAS with T4 at the thyroid hormone transport protein TTR.
- **Sensitivity (LOD/Q):** depends on sample amount and matrix.
- **Variability (e.g. CV for single substance tests) if known**: <20%.
- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the extraction/clean-up as well as which kinds of matrixes.
- **Complexity/learning period**: 1 week of training.

- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** BDS.
- **Water framework Directives:** locally in first evaluation projects.
- **Publications:** please ask for more info our sales representative**.**

Typical applications for PFAS CALUX test




p53 CALUX (Catalogue No. 11)

The p53 CALUX® is a human cell line (U2OS) derived pathway selective reporter gene assay. In this assay, a firefly luciferase gene has been coupled to p53 Responsive Elements. The luciferase serves as a reporter gene for the presence p53-pathway activating compounds. The pathway is activated by genotoxic compounds that do not require metabolic activation and in rare cases agents that induce cell cycle arrest. Activation of the p53-pathway will lead to luciferase expression, and by addition of the appropriate substrate for luciferase, this can be detected as light. The amount of light produced is proportional to the concentration of p53-pathway activating compounds.

- **Endpoint (unit):** positive or negative for p53 activation/amount processed or dilution factor.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: ECVAM method DB-ALM Protocol n° 197.: Automated CALUX reporter gene assay procedure.
- **Positive control used**: Actinomycin D.
- **Matrices (sediment, water, tissue etc.) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human osteosarcoma cell line U2OS.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed.
- **Current use on MS level (regular monitoring as well as other purposes):** Several R&D projects.
- **Assessment criteria:** typical performance criteria from Dutch Rijkswaterstaat RIKZ-Specie-08 guideline can be used.
- **Specificity:** many genotoxic compounds.
- **Sensitivity (LOD/Q):** N/A.
- **Variability (e.g., CV for single substance tests) if known:** <20%.
- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as which kind of water matrixes.

- **Complexity/learning period:** 1 week of training.
- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** BDS.
- **International relevance:** applied in several projects.
- **Publications:**

Van der Linden, SC, von Bergh A, Van Vugt-Lussenburg B, Jonker L, Brouwer A, Teunis M, Krul C and Van der Burg B. Development of a panel of high throughput reporter gene assays to detect genotoxicity and oxidative stress, Mutation Res., in press.

(please see for more info at www.bds.nl under literature)

Typical applications for all P53 CALUX tests

P53 + S9 CALUX (Catalogue No. 12)

The genotox CALUX® is a human cell line (U2OS) derived pathway selective reporter gene assay. In this assay, a firefly luciferase gene has been coupled to p53 Responsive Elements. The luciferase serves as a reporter gene for the presence p53-pathway activating compounds. Activation of the p53-pathway will lead to luciferase expression, and by addition of the appropriate substrate for luciferase, this can be detected as light. The amount of light produced is proportional to the concentration of p53-pathway activating compounds. The pathway is activated by many genotoxic compounds and in rare cases agents that induce cell cycle arrest. Because many genotoxic compounds exert their genotoxic effect only after they have been activated by metabolic enzymes, the genotox CALUX® is performed in both the absence and the presence of metabolic enzyme-containing rat liver S9 mix. In addition, a protocol is used that has been validated using a panel of genotoxic and non-genotoxic agents, showing high specificity and sensitivity.

- **Endpoint (unit):** positive or negative for genotoxicity/amount processed or dilution factor.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: none.
- **Positive control used**: Cyclophosphamide.
- **Matrices (sediment, water, tissue etc.) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human osteosarcoma cell line U2OS.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed.
- **Current use on MS level (regular monitoring as well as other purposes):** Several R&D projects.
- **Assessment criteria:** typical performance criteria from Dutch Rijkswaterstaat RIKZ-Specie-08 guideline can be used.
- **Specificity:** most genotoxic compounds.
- **Sensitivity (LOD/Q):** N/A.
- **Variability (e.g,. CV for single substance tests) if known:** <20%.

- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as which kinds of water matrixes.
- **Complexity/learning period:** 1 week of training.
- **Costs:** Costs are generally not depending on matrix studied.
- **Commercial availability:** BDS.
- **International relevance:** applied in several projects.
- **Publications:**

Van der Linden, SC, von Bergh A, Van Vugt-Lussenburg B, Jonker L, Brouwer A, Teunis M, Krul C and Van der Burg B. Development of a panel of high throughput reporter gene assays to detect genotoxicity and oxidative stress, Mutation Res., in press.

(please see for more info at www.bds.nl under literature)

Typical applications for p53 + S9 CALUX

GENERAL TOXICITY TESTING

Cytotox CALUX (Catalogue No. 013)

The Cytotox CALUX® consists of a human osteosarcoma cell line (U2OS) that constitutively expresses a high level of luciferase. By addition of the appropriate substrate for luciferase, light is emitted. If the cells are exposed to cytotoxic compounds as a result the amount of luciferase expressed will decrease. This can be measured as a decrease in the light signal. As such the line is also used as a generic control in CALUX assay panels. The Cytotox CALUX reports whether a sample is cytotoxic, and at which concentration or dilution factor the cytotoxicity occurs.

- **Endpoint (unit):** positive or negative for genotoxicity/amount processed or dilution factor.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: ECVAM method DB-ALM Protocol n° 197.: Automated CALUX reporter gene assay procedure.
- **Positive control used**: none.
- **Matrices (sediment, water, tissue etc.) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human osteosarcoma cell line.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ). Use of 1 liter water.
- **Current use on MS level (regular monitoring as well as other purposes):** Several R&D projects.
- **Assessment criteria:** typical performance criteria from Dutch Rijkswaterstaat RIKZ-Specie-08 guideline can be used.
- **Specificity:** many cytotoxic compounds.
- **Sensitivity (LOD/Q):** N/A.
- **Variability (e.g., CV for single substance tests) if known:** <20%.

- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as the kind of matrix.
- **Complexity/learning period:** 1 week of training.
- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** BDS.
- **International relevance:** applied in several projects.
- **Publications:**

Van der Linden, SC, von Bergh A, Van Vugt-Lussenburg B, Jonker L, Brouwer A, Teunis M, Krul C and Van der Burg B. Development of a panel of high throughput reporter gene assays to detect genotoxicity and oxidative stress, Mutation Res., in press.

Please see for more info at www.bds.nl under literature

Typical applications for Cytotox CALUX test

Relevant for all application fields

Nrf2 oxidative stress CALUX (Catalogue No. 14)

The Nrf2 Responsive (Nrf2) CALUX® is composed of a human cell line (U2OS) containing the firefly luciferase gene uder control of four Electrophile Responsive Elements (EpREs). The luciferase serves as a reporter gene for activation of the Nrf2 pathway. This pathway is activated by oxidative stress and antioxidants. Activation of the pathway will lead to luciferase expression, and by addition of the appropriate substrate for luciferase, this can be detected as light. The amount of light produced is proportional to the concentration of the Nrf2-pathway activating compounds. The pathway activation caused by the sample is compared to the activation elicited by the positive control, curcumine. Nrf2 CALUX bioassays report total curcumine equivalents.

- **Endpoint (unit):** pg curcumine equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: ECVAM method DB-ALM Protocol n° 197.: Automated CALUX reporter gene assay procedure.
- **Positive control used**: curcumine.
- **Matrices (sediment, water, tissue etc) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human osteosarcoma cell line.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ).
- **Current use on MS level (regular monitoring as well as other purposes):** Several R&D projects.
- **Assessment criteria:** typical performance criteria from Dutch Rijkswaterstaat RIKZ-Specie-08 guideline can be used.
- **Specificity:** oxidation of the Nrf2 repressing protein Keap1.
- **Sensitivity (LOD/Q):** N/A.
- **Variability (e.g., CV for single substance tests) if known:** <20%.

- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as the kind of matrix. The use of the cytotox CALUX line is also used as a generic control in CALUX assay panels.

- **Complexity/learning period:** 1 week of training.

- **Costs:** Costs are generally depending on matrix studied.

- **Commercial availability:** BDS.

- **International relevance:** applied in several projects. Published trigger value of 3 µg CurEQ/ water (van der Oost et al. 2017).

- **Publications:**
  Van der Linden, SC, von Bergh A, Van Vugt-Lussenburg B, Jonker L, Brouwer A, Teunis M, Krul C and Van der Burg B. Development of a panel of high throughput reporter gene assays to detect genotoxicity and oxidative stress, Mutation Res., in press.

  Please see for more info at www.bds.nl under literature.

Typical applications for Nrf2 CALUX test

RARα CALUX (agonistic/antagonistic) (Catalogue No. 015)

The RARα Responsive (RARα) CALUX® comprise a human bone marrow cell lines (U2OS), incorporating the firefly luciferase gene coupled to RARα Responsive Elements (RARα REs) as a reporter gene for the presence of RARα -like compounds. Following binding of RARα -like compounds to the cytosolic RARα receptor, the ligand-receptor complex binds the RARα RE. Cells that are exposed to RARα -like compounds not only express proteins that are under normal circumstances associated to RARα RE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds all trans retinoic acids. RARα CALUX bioassays report total all trans retinoic acids equivalents for environmental matrices.

- **Endpoint (unit):** pg all trans retinoic acids equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: new.
- **Positive control used**: all trans retinoic acids.
- **Matrices (sediment, water, tissue etc.) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human bone marrow cell line U2OS.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ). For 1 liter of water is processed resulting in a LOQ of ca. 10 pg all trans retinoic acids equivalents per liter of water, respectively.
- **Current use on MS level (regular monitoring as well as other purposes):** Several Dutch R&D projects
- **Assessment criteria:** typical performance criteria from Dutch Rijkswaterstaat RIKZ-Specie-08 guideline can be used.
- **Specificity:** Binding to the RARα receptor.
- **Sensitivity (LOD/Q):** RARα CALUX: of ca. 10 pg all trans retinoic acids equivalents per liter of water respectively.

- **Variability (e.g., CV for single substance tests) if known:** <20%.
- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as which kinds of water matrixes.
- **Complexity/learning period:** 1 week of training.
- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** BDS.
- **International relevance:** applied in several projects.
- **Publications:** please see for more info at www.bds.nl under literature.

Typical applications for RARα CALUX test

ERSE (endoplasmic reticulum stress) CALUX (Catalogue No. 16)

The endoplasmic reticulum (ER) is the cell organelle responsible for proper protein folding in eukaryotic species. This process may be disrupted by a various diseases and chemical compounds, resulting in accumulation of unfolded or misfolded proteins. The mammalian response towards this type of stress is mediated by the ERSE-element in the promoters of ER-stress responsive genes. The ERSE CALUX is a human cell line based (U2OS) reporter in which luciferase expression is mediated by a minimal promoter of multimerized ERSE elements. This production may be quantified by addition of the appropriate substrate for luciferase, resulting in the production of an amount of light proportional to the stress level. The response is expressed relative to the response towards the reference compound tunicamycin, which is an established reference compound for disruption of protein folding.

- **Endpoint (unit):** pg tunicamycin equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: new.
- **Positive control used**: tunicamycin.
- **Matrices (sediment, water, tissue etc) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human osteosarcoma cell line.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ).
- **Current use on MS level (regular monitoring as well as other purposes):** Several R&D projects.
- **Assessment criteria:** typical performance criteria from Dutch Rijkswaterstaat RIKZ-Specie-08 guideline can be used.
- **Specificity:** activation of the ERSE pathway.
- **Sensitivity (LOD/Q):**
- **Variability (e.g.; CV for single substance tests) if known:** <20%.

- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as the kind of matrix. The use of the cytotox CALUX line is also used as a generic control in CALUX assay panels.
- **Complexity/learning period:** 1 week of training.
- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** BDS.
- **International relevance:** applied in several projects.

Typical applications for ERSE CALUX

LXR (Liver X receptors) CALUX (agonistic / antagonistic)

(Catalogue No. 17)

Liver X receptors play a dominant role in processes that relate to cholesterol, fatty acid and glucose homeostasis. For this reason, their activity could play a role in metabolic disorders. Activated LXR binds to the LXR response element (LXRE) of its target genes. The LXR CALUX is a human cell line based (U2OS) reporter in which luciferase expression is mediated by a minimal promoter of multimerized LXRE elements. This production may be quantified by addition of the appropriate substrate for luciferase, resulting in the production of an amount of light proportional to the LXR-induced cellular activity. The response is expressed relative to the response towards the synthetic LXR agonist GW3965.

- **Endpoint (unit):** pg GW3965 equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: new.
- **Positive control used**: GW3965.
- **Matrices (sediment, water, tissue etc.) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human osteosarcoma cell line.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ).
- **Current use on MS level (regular monitoring as well as other purposes):** Several R&D projects.
- **Assessment criteria:** typical performance criteria from Dutch Rijkswaterstaat RIKZ-Specie-08 guideline can be used.
- **Specificity:** binding to LXR.
- **Sensitivity (LOD/Q):** N/A.
- **Variability (e.g., CV for single substance tests) if known:** <20%.

- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as the kind of matrix. The use of the cytotox CALUX line is also used as a generic control in CALUX assay panels.
- **Complexity/learning period:** 1 week of training.
- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** BDS.
- **International relevance:** applied in several projects.
- **Publications:** please see for more info at www.bds.nl under literature.

Typical applications for LXR CALUX

Hif-1 (hypoxia response) CALUX (Catalogue No. 18)

Hypoxia inducible factors are regulatory protein complexes of which the levels build up in mammalian cells when oxygen availability in the cell becomes limited. Under normal condition, Hif-1 is degraded. Hif-1 has an established role in growth, development, energy metabolism and angiogenesis. In addition to real hypoxia, hif-1 also responds to various chemicals that initiate a mimic of the hypoxic response. hif-1 complex binds to the hypoxic response element (HRE) of its target genes. The hif-1 CALUX is a human bone marrow cell line-based (U2OS) reporter in which luciferase expression is mediated by a minimal promoter of multimerized HRE elements. This production may be quantified by addition of the appropriate substrate for luciferase, resulting in the production of an amount of light proportional to the hif-1 -induced cellular activity. The response is expressed relative to the response towards cobaltous chloride.

- **Endpoint (unit):** pg cobaltous chloride equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: new.
- **Positive control used**: cobaltous chloride.
- **Matrices (sediment, water, tissue etc) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human osteosarcoma cell line.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ).
- **Current use on MS level (regular monitoring as well as other purposes):** Several R&D projects.
- **Assessment criteria:** typical performance criteria from Dutch Rijkswaterstaat RIKZ-Specie-08 guideline can be used.
- **Specificity:** activation of the Hif-1 pathway.
- **Sensitivity (LOD/Q):** N/A.
- **Variability (e.g., CV for single substance tests) if known:** <20%

- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as the kind of matrix. The use of the cytotox CALUX line is also used as a generic control in CALUX assay panels.
- **Complexity/learning period:** 1 week of training
- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** BDS
- **International relevance:** applied in several projects.
- **Publications:** please see for more info at www.bds.nl under literature.

Typical applications for HIF-1 CALUX

AP1 (activator protein 1) CALUX (Catalogue No. 19)

Activator protein 1 (AP1) is a regulatory protein complex that is involved in the regulation of proliferation, differentiation, and apoptosis. Depending on the circumstances AP1 can exert oncogenic or anti-oncogenic effects.

The AP1 CALUX is a human cell line based (U2OS) reporter in which luciferase expression is mediated by a minimal promoter of multimerized TPA response elements (TREs). This production may be quantified by addition of the appropriate substrate for luciferase, resulting in the production of an amount of light proportional to the AP1 - induced cellular activity. The response is expressed relative to the response towards tetradecanoyl phorbol acetate (TPA).

- **Endpoint (unit):** pg tetradecanoyl phorbol acetate equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: new.
- **Positive control used**: tetradecanoyl phorbol acetate.
- **Matrices (sediment, water, tissue etc) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human osteosarcoma cell line.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ).
- **Current use on MS level (regular monitoring as well as other purposes):** Several R&D projects.
- **Assessment criteria:** typical performance criteria from Dutch Rijkswaterstaat RIKZ-Specie-08 guideline can be used.
- **Specificity:** activation of the AP1 pathway.
- **Sensitivity (LOD/Q):** N/A/
- **Variability (e.g., CV for single substance tests) if known:** <20%.

- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as the kind of matrix. The use of the cytotox CALUX line is also used as a generic control in CALUX assay panels.
- **Complexity/learning period:** 1 week of training.
- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** BDS.
- **International relevance:** applied in several projects.
- **Publications:** please see for more info at www.bds.nl under literature.

Typical applications for AP-1 CALUX

For more information's, please visit also our webpage www.bds.nl.

PPARα CALUX (agonistic/antagonistic) (Catalogue No. 020)

The PPARα Responsive (PPARα) CALUX® comprise a human bone marrow cell lines (U2OS), incorporating the firefly luciferase gene coupled to PPARα Responsive Elements (PPARα REs) as a reporter gene for the presence of PPARα -like compounds. Following binding of PPARα -like compounds to the cytosolic PPARα receptor, the ligand-receptor complex binds the PPARα RE. Cells that are exposed to PPARα -like compounds not only express proteins that are under normal circumstances associated to PRE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compound Rosiglitazone. PPARα CALUX bioassays report total Rosiglitazone equivalents per unit of sample matrix.

- **Endpoint (unit):** pg Rosaglitazone equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: ECVAM method DB-ALM Protocol n° 197.: Automated CALUX reporter gene assay procedure.
- **Positive control used**: Rosaglitazone.
- **Matrices (sediment, water, tissue etc.) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human bone marrow cell line U2OS.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ). The bioassays' LOQ is ca. 1 pg Rosaglitazone equivalents per amount of material processed. For 1 liter of water is processed resulting in a LOQ of ca. 1 pg Rosaglitazone equivalents per liter of water, respectively.
- **Current use on MS level (regular monitoring as well as other purposes):** new endpoint.
- **Assessment criteria:** typical performance criteria from Dutch Rijkswaterstaat RIKZ-Specie-08 guideline can be used.
- **Specificity:** Binding to the PPARα receptor.

- **Sensitivity (LOD/Q):** Original PPARα CALUX: ca.1 pg Rosaglitazone /l water.
- **Variability (e.g., CV for single substance tests) if known:** <20%.
- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as which kinds of water matrixes.
- **Complexity/learning period:** 1 week of training.
- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** BDS.

- **International relevance:** PFAAs (like PFOA) are binding to PPAR. Test just recently developed and yet not often applied.
- **Publications:** please see for more info at www.bds.nl under literature.

PPARδ CALUX (agonistic/antagonistic) (Catalogue Nr. 021)

The PPARδ Responsive (PPARδ) CALUX® comprise a human bone marrow cell lines (U2OS), incorporating the firefly luciferase gene coupled to PPARδ Responsive Elements (PPARα REs) as a reporter gene for the presence of PPARδ -like compounds. Following binding of PPARδ -like compounds to the cytosolic PPARδ receptor, the ligand-receptor complex binds the PPARα RE. Cells that are exposed to PPARδ -like compounds not only express proteins that are under normal circumstances associated to PRE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds Rosaglitazone. PPARδ CALUX bioassays report total Rosaglitazone equivalents for environmental matrices.

- **Endpoint (unit):** pg Rosaglitazone equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: ECVAM method DB-ALM Protocol n° 197.: Automated CALUX reporter gene assay procedure.
- **Positive control used**: Rosaglitazone.
- **Matrices (sediment, water, tissue etc.) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human bone marrow cell line U2OS.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ). The bioassays' LOQ is ca. 1 pg Rosaglitazone equivalents per amount of material processed. For 1 liter of water is processed resulting in a LOQ of ca. 1 pg Rosaglitazone equivalents per liter of water, respectively.
- **Current use on MS level (regular monitoring as well as other purposes):** new endpoint.
- **Assessment criteria:** typical performance criteria from Dutch Rijkswaterstaat RIKZ-Specie-08 guideline can be used.
- **Specificity:** Binding to the PPARα receptor.

- **Sensitivity (LOD/Q):** Original PPARα CALUX: ca.1 pg Rosaglitazone /l water.
- **Variability (e.g., CV for single substance tests) if known:** <20%.
- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as which kinds of water matrixes.
- **Complexity/learning period:** 1 week of training.
- **Costs:** Costs are generally depending on matrix studied.
- **Commercial availability:** BDS.
- **International relevance:** PFAAs (like PFOA) are binding to PPAR. Test just recently developed and yet not often applied.
- **Publications:** please see for more info at www.bds.nl under literature.

PPARγ CALUX (agonistic/antagonistic) (Catalogue Nr. 022)

The PPARγ Responsive (PPARα) CALUX® comprise a human bone marrow cell lines (U2OS), incorporating the firefly luciferase gene coupled to PPARγ Responsive Elements (PPARγ REs) as a reporter gene for the presence of PPARγ -like compounds. Following binding of PPARα -like compounds to the cytosolic PPARγ receptor, the ligand-receptor complex binds the PPARγ RE. Cells that are exposed to PPARγ -like compounds not only express proteins that are under normal circumstances associated to PRE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds Rosaglitazone. PPARγ CALUX bioassays report total Rosaglitazone equivalents for environmental matrices.

- **Endpoint (unit):** pg Rosaglitazone equivalents/g sample processed.
- **Test duration:** 24h.
- **Guidelines and/or standards (international, such as ISO, CEN; or national such as DIN) available (if not available, please refer to publication with protocol)**: ECVAM method DB-ALM Protocol n° 197.: Automated CALUX reporter gene assay procedure.
- **Positive control used**: Rosaglitazone.
- **Matrices (sediment, water, tissue etc) that can be investigated:** Any type of sample.
- **Tissue/cells examined:** Human bone marrow cell line U2OS.
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ). The bioassays' LOQ is ca. 15 pg Rosaglitazone equivalents per amount of material processed. For 1 liter of water is processed resulting in a LOQ of ca. 15 pg Rosaglitazone equivalents per liter of water, respectively.
- **Current use on MS level (regular monitoring as well as other purposes):** new endpoint.
- **Assessment criteria:** typical performance criteria from Dutch Rijkswaterstaat RIKZ-Specie-08 guideline can be used.
- **Specificity:** Binding to the PPARγ receptor.
- **Sensitivity (LOD/Q):** Original PPARγ CALUX: ca.20 pg Rosaglitazone /l water.

- **Variability (e.g., CV for single substance tests) if known:** <20%.
- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as which kinds of water matrixes.

- **Complexity/learning period:** 1 week of training

- **Costs:** Costs are generally depending on matrix studied.

- **Commercial availability:** BDS.

- **International relevance:** PFAAs (like PFOS) are binding to PPAR.

  Elevated levels found in NL and CH. Published trigger value of 10 ng RosEQ/ l water (van der Oost et al 2017).

- **Publications:** please see for more info at www.bds.nl under literature

Typical applications for all PPAR CALUX tests



For more information's regarding publications of our partners and us by using CALUX technologies, please also visit our library/literature data base at our webpage at http://www.biodetectionsystems.com/1/sub/22.php

For more case-by-case studies and applications and/or presentations of CALUX users, please also visit our webpage based down-loadable database of the last 12 BioDetectors conferences at e.g., http://www.biodetectionsystems.com/1/news/105.php