

BioDetection Systems

CALUX[®] Assays

PAH CALUX[®] technology

BDS' PAH CALUX[®] the bioassay

BDS' receptor mediated **Poly-Aromatic Hydrocarbon (PAH) Responsive Chemically Activated Luciferase eXpression** bioassay (**PAH CALUX[®]**) is the perfect screening tool for the detection of Benzo(a)pyrene and other PAH-like chemicals.

Why use BDS' PAH CALUX[®] bioassay?

- Much reduced cost compared to instrumental methods (luminometer measurement below 100 Euro/sample)
- Rapid using a fast sample preparation and a 96 well plate format (just in 8 hrs)
- Extremely sensitive (a few pg/gr fat or pg/gr matrix)
- Easy sample clean-up / work-up (BDS SOPs)
- Small sample size (1 to 5 gr)
- Applicable to a wide variety of matrices (see next pages).
- Excellent correlations (e.g. $R^2 \geq 0.96$ for B(k)F) to several PAH's already published

Who uses BDS' PAH CALUX[®] bioassay?

- Feed/Food Industry according to EU guideline 2006/1881/EG
- Chemical Industry – testing of active compounds
- Governmental Monitoring projects for sediments and water used such in vitro reporter gene assays
- Monitoring studies for human blood, urine and milk
- “Face it” 2005-2008: Biological Risk assessment of oils spills – establishment of a rapid alert system
- First evaluation of PAH determination by PAH CALUX in smoked pork has been published by Kuhn et al (2008) in the Journal of Food Protection Vol. 71, No.5, pp 993.

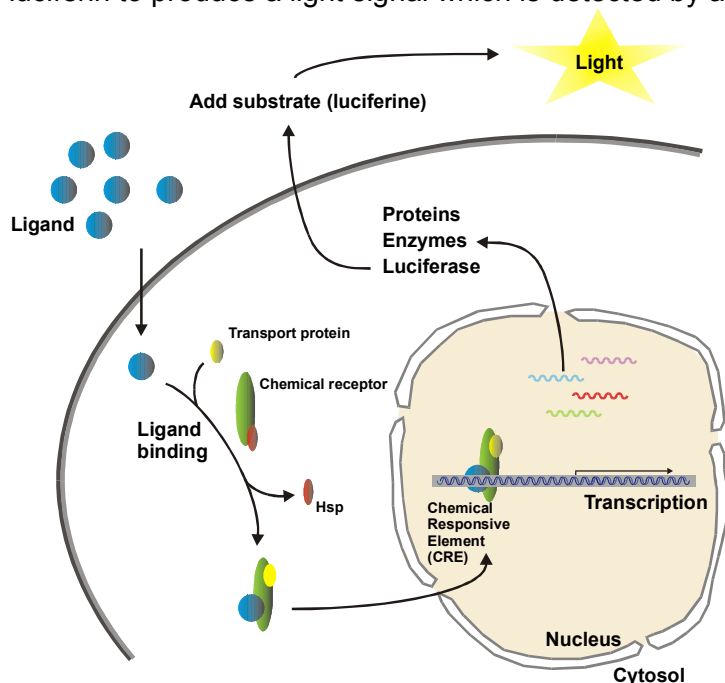


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CALUX[®] Method

BDS has constructed a series of bioassays for the detection of a range of groups of potent chemical contaminants. These receptor mediated Chemically Activated Luciferase eXpression or CALUX[®] bioassays have proven to be powerful analytical tools with broad applications.

All of the CALUX[®] bioassays operate using the same basic core principle. Mammalian cells (rat liver H4IIE cells) are selected that contain receptors that are involved in the toxicological mode of action of the groups of chemicals of interest. In the normal cell state, when the cells are exposed to these chemicals, binding at the relevant receptor occurs resulting in the production of a receptor-ligand complex. This receptor-ligand complex in turn translocates to the cell nucleus where it interacts with specific DNA sequences triggering gene expression and the synthesis of proteins/enzymes which are implicated in the toxicological effect observed. In CALUX[®] cells, the firefly gene which codes for the synthesis of the enzyme luciferase is incorporated into the DNA of the CALUX[®] cell nucleus. The luciferase gene is the gene which gives the firefly the ability to produce light. This incorporation is done in such a way so that when binding of the receptor-ligand complex takes place on the DNA, in addition to the normal triggering of gene expression described above, the luciferase gene is activated and luciferase is produced. The amount of luciferase produced is proportional to the extent of receptor binding and it is quantitated by reaction with luciferin to produce a light signal which is detected by a luminometer.



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CALUX[®] Application

Performance Properties of the DR CALUX[®] bioassay:

✓ *Extremely sensitive*

BDS' PAH CALUX[®] bioassay is capable of detecting picograms (10^{-12} grams) of Benzo(a)pyren equivalents (EQs). The method limit of quantification of the bioassay is ca. 6.2 pg BaP-EQ / well. To convert this to more useful information, Limits of Detection (LOD) and Limits of Quantitation (LOQ) in a variety of example matrices are given in the table below:

LOD and LOQ in the PAH CALUX[®] bioassay for some example matrices

Matrix	Material processed	BaP EU limits (µg/kg wet weight)	LOQ	Units
Milk	1.5 g fat	1.0	60	BaP-EQ pg/g fat
Vegetable oil	5 g fat	2.0	20	BaP-EQ pg/g fat
Sediment	5 g	no	20	BaP-EQ pg/g sediment

List of matrices for which the PAH CALUX[®] bioassay can be used

Food and Feed samples	Environmental samples	Water samples	Tissue samples	Other
Animal Feedstuffs	Soils / clays	Surface water	Animal tissues	Pure chemicals (pharmaceuticals, agrochemicals, fine chemicals, etc.)
Plant/vegetable matter	Stack samples	Groundwater	Human tissues	
Minerals	Air samples	Drinking water	Blood (plasma; serum)	
Animal fat	Sediments	Wastewater		
Meat		Effluents		
Chicken		Sewage[sludge]		
Veg. Oils				
Egg				
Fish				
Milk				
Fishoil				

Note: Other matrices may be processed directly or may require extraction development. Please contact us to discuss the procedures for specific matrices of your interest.

✓ *BDS' DR CALUX[®] bioassay....the basis of European law for Benzo(a)pyrene analysis using bioassays*

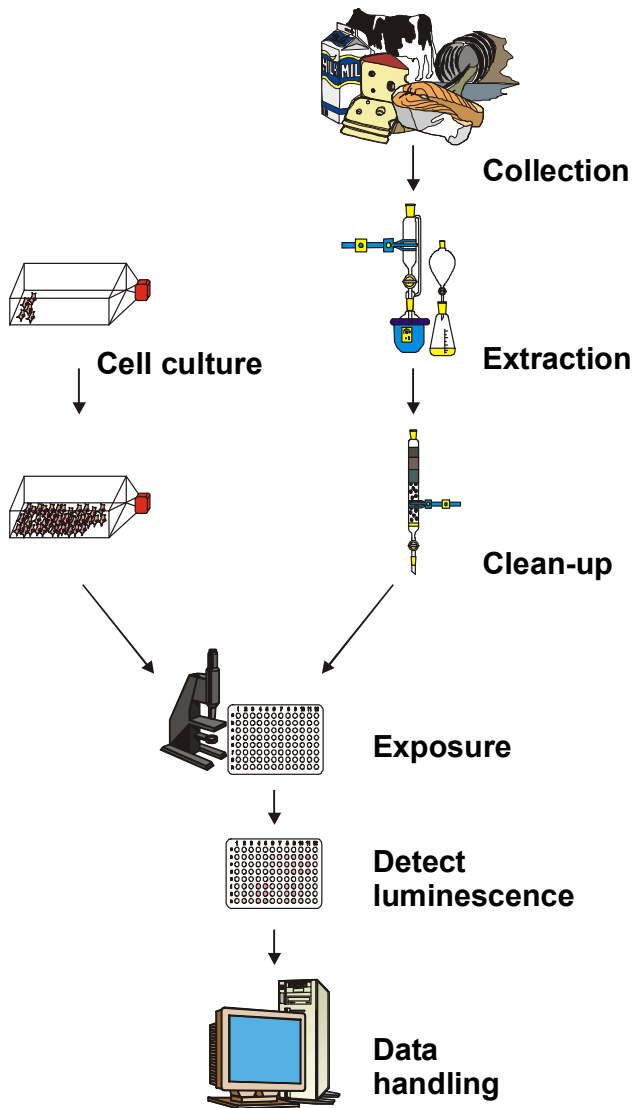
In Europe, most of the member countries have now fully brought EU Directives (2006/1881/EC) into national law and thus limit values for BaP in food and feed are now a compliance matter. **Any food or feed imported into the EU must comply with the BaP limit values.**

✓ *Distinguish between stable and non-stable PHAHs or PAHs*

The PAH CALUX[®] bioassay can be used to distinguish between stable and non-stable PHAHs or PAHs by variation of the time of exposure.



BioDetection Systems *CALUX*[®] Analysis



1) Sample collection: Sampling procedures are dependent on the matrix of interest. Samples should be handled with care and typically packed in glass vials or bottles with a PTFE or Teflon lined caps.

2) Extraction: Extraction procedures depend on the matrix of interest. Typical extraction techniques used are accelerated (ASE) and 2-phase solvent extraction.

3) Clean-up: If required, a clean-up procedure is used on extracts to reduce fat.

4) Exposure: Cells are cultivated under standardised conditions in culture flasks and prior to exposure transferred to 96-well plates. Cells are exposed to cleaned-up extracts diluted in DMSO. Exposures are performed in triplicate and last typically for 6 hrs up to 24 hrs. After exposure, the cells are lysed and luciferin (substrate for luciferase) is added.

5) Quantification: Luciferase activity produced is proportional to the amount of material of interest present and is determined using a luminometer.

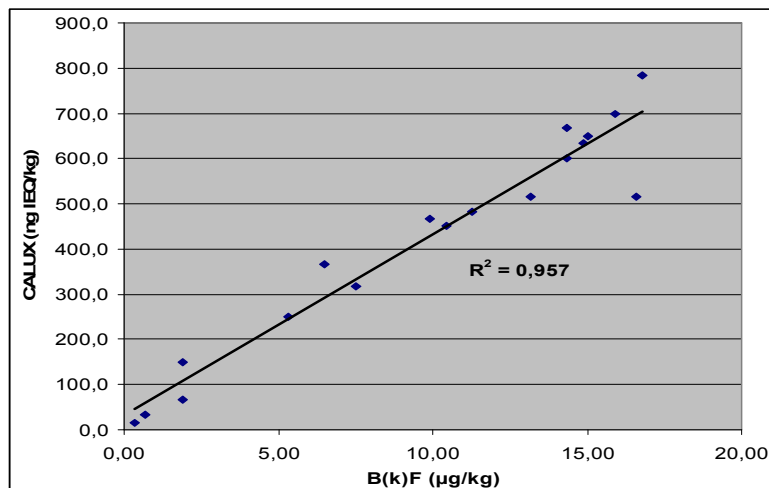
6) Data handling: On each 96-well plate, cells are exposed to a full reference material concentration range (also in triplicate). The emitted light from these exposures is used to construct a reference material calibration curve. The calibration curve is then used to determine the test material TEQ (usually in pg BaP-EQ/g per sample) content for the individual samples tested

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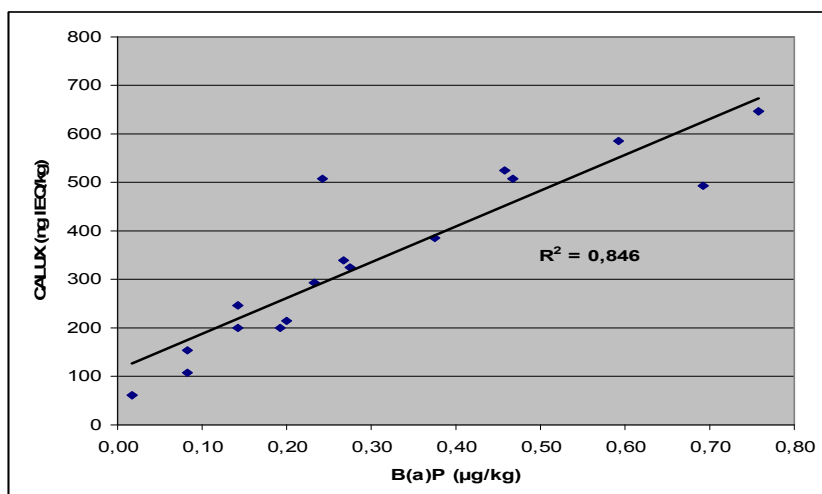
PAH CALUX[®] technology

BDS' PAH CALUX[®] versus chemical analysis

- A.) Comparison benzo(k)fluoranthene and CALUX bioassay for 20 smoked pork belly samples measured in induction equivalents (IEQ) from Kuhn et al. (2008) in Journal of Food Protection Vol 71, No. 5, pages 993-999.



- B.) Comparison benzo(a)pyrene and CALUX bioassay for 20 smoked pork belly samples measured in induction equivalents (IEQ) from Kuhn et al. (2008) in Journal of Food Protection Vol 71, No. 5, pages 993-999.



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Benzo(a)pyrene- Equivalent Factors

PAH	log(EC50)	IARC class	Rel potency (at EC50)
Naphtalene	No binding or >-4	-	<0.001
Acenaphthylene	No binding or >-4	-	<0.001
Acenaphthene	No binding or >-4	3	<0.001
Fluorene	No binding or >-4	3	<0.001
Phenanthrene	No binding or >-4	3	<0.001
Anthracene	No binding or >-4	3	<0.001
Fluoranthene	No binding or >-4	3	<0.001
Pyrene	No binding or >-4	3	<0.001
Chrysene	-8.4	2B	0.84
Benzo(a)pyrene	-8.5	1	1
Benzo(k)fluoranthene	-9.0	2B	3.7
Indeno (1,2,3-c,d)pyrene	-8.6	2B	1.3
Benzo(g,h,i)perylene	No binding or >-4	3	<0.001
Benzo[c]phenanthrene	No binding or >-4	2B	<0.001
Benzo(a)anthracene	-8.1	2B	0.42
Benzo(b)fluoranthene	-9.6	2B	13.9
Dibenzo(a,h)anthracene	-8.7	2A	1.7



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BDS' PAH CALUX[®] the bioassay

Typical EC 50 curves of selected PAHs

