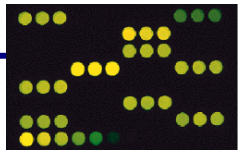
HIYOSHI
Ecological Services

CALUX® Assay (by XDS)

rapid screening method for Dioxins



Introduction

CALUX Assay (**C**hemically **A**ctivated **Lu**ciferase E**x**pression) is an **inexpensive** and **rapid** screening assay for detecting dioxin-like compounds (PCDDs, PCDFs, and DL-PCBs) which has high correlation with the traditional method, HRGC/HRMS. It is a unique technique developed by an American company, Xenobiotic Detection Systems, Inc (XDS) and was awarded a patent in December, 1998 in the United States of America.

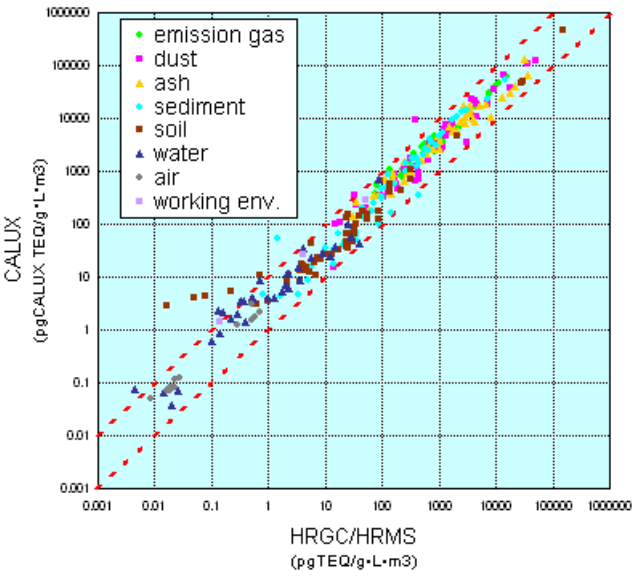
Hiyoshi began a joint study with XDS in 1998 and came to a licensing agreement in 2000. Since then, Hiyoshi is working to promote the CALUX Assay in Japan and in Asia. Meanwhile, we have done many joint studies with governmental institutes, universities and private companies to prove the superiority of the CALUX Assay.

CALUX Assay can be used for dioxin analysis of environmental matrices such as soil, sediment, ash, water, exhaust gas, as well as biological such as blood, breast milk, fatty tissue, and food matrix as fish or daily products.

Feature

Our CALUX Assay has a high accuracy of analysis of dioxin-like compounds and analysis range at the same level as the instrumental analysis method. In addition, it has following merits listed below.

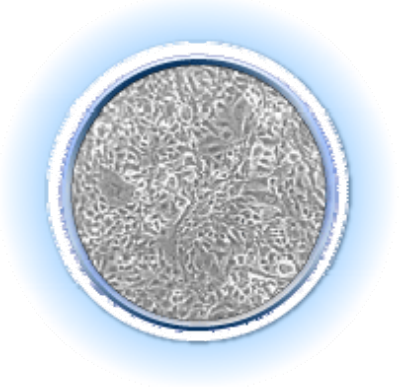
	HRGCMS	CALUX
Turnaround	1.5month	**3-5days**
Sample amount	50g	**2 ～10g**
Speed of Analysis	1sample/hr	**16samples/hr**



Mechanism of CALUX Cell

The recombinant cell line used in this assay (H1L6.1c2) was generated by stably transfecting the plasmid pGudLuc6.1 into mouse hepatoma (Hepa1c1c7) cells. The pGudLuc6.1 plasmid contains the CYP1A1 dioxin-responsive domain (inclusive of four DREs) upstream of the firefly luciferase gene.

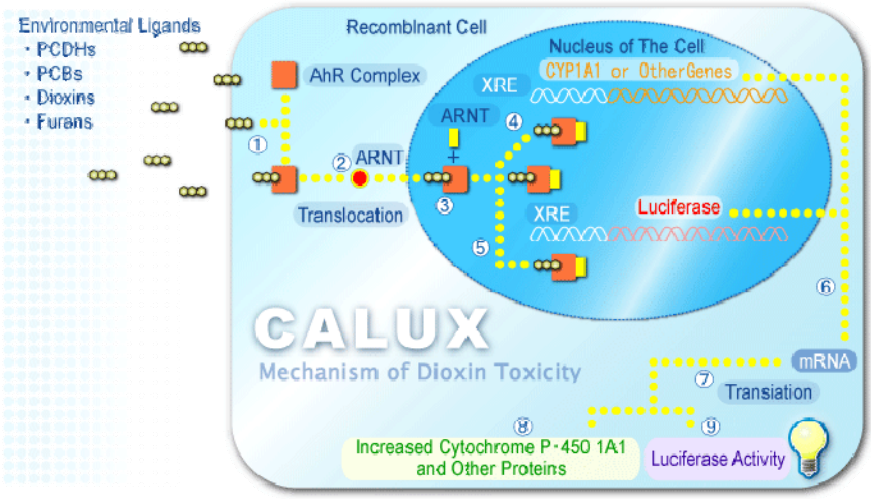
The recombinant mouse hepatoma cell line, Hepa1c1c7



(introduction of dioxin toxicity)

① Polychlorinated diaromatic hydrocarbons (PCDH), including dioxins bind to an intracellular receptor called the aryl hydrocarbon receptor (Ah Receptor) and activate the receptor.

② The PCDH-Ah Receptor complex then travels to the nucleus of the cell.



③ Activated PCDH-Ah Receptor then binds to specific sequences in the DNA called dioxin responsive elements (DRE).

④ The binding of the PCDH-Ah Receptor complex to the DRE causes the expression of the associated genes to be transcript and altered.

⑤ Synthesizing of luciferase is directed.

⑥ The messenger RNA (mRNA) then transfer to cytoplasm.

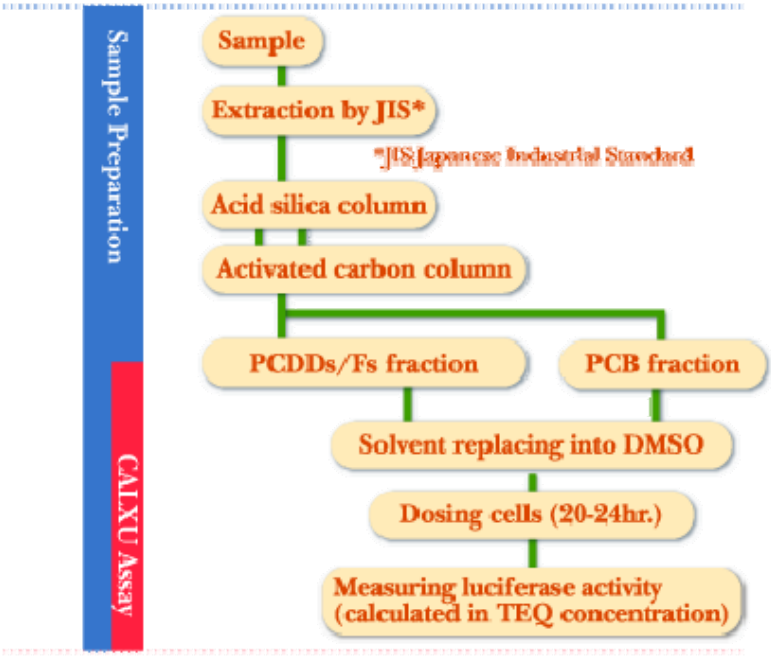
⑦ mRNA translate to polypeptides in cytoplasm.

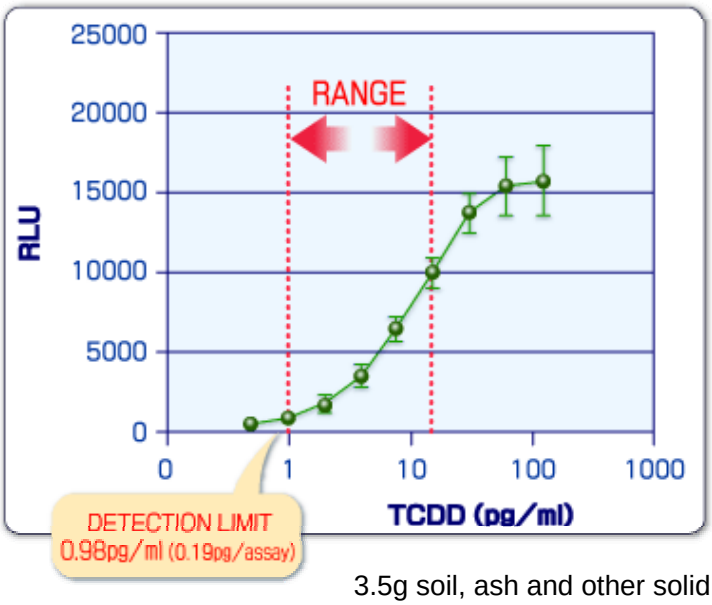
⑧ New proteins will be synthesized from the polypeptides. It is this protein that causes the toxic effects that are observed.

⑨ Dioxin TEQ is measured from luminescence produced by the luciferase reporter gene.

Summary of CALUX Assay Procedure

First, samples will be extracted following JIS (Japanese Industrial Standard) or by original sonication extraction method. Concentrate the rough extract and re-suspend it in hexane then treat it with XDS patented clean-up method. Apply the sample to acid silica column and XCARB column (activated carbon column) and extract Co-PCB and PCDD/Fs using appropriate solvent (There are cases of fractioning not necessary). Replace the sample solvent into DMSO and dose H1L1.6 mouse hepatoma cell grown in 96 well plate with the sample. Meanwhile add dilution series of 2,3,7,8-TCDD to the same plate for standard curve. After 24 hours of dosing, measure luciferase amount that is generated relative to dioxin concentration and calculate total CALUX TEQ.





- DETERMINATION LIMIT : 0.098pgTEQ/well
- DETECTION LIMIT : 0.19pgTEQ/well
- UPPER DETECTION LIMIT:90 LIMIT(Test Experiment 0.00pgTEQ/well)
- DETECTION RANGE : 0.98pg/ml ~ 15.6pg/ml

3.5g soil, ash and other solid sample: 1.0pgTEQ/g
3.5L water and other liquid samples: 1.0pgTEQ/L
3.5m^3N exhaust gas and other gas samples: 1.0pg TEQ/m^3N

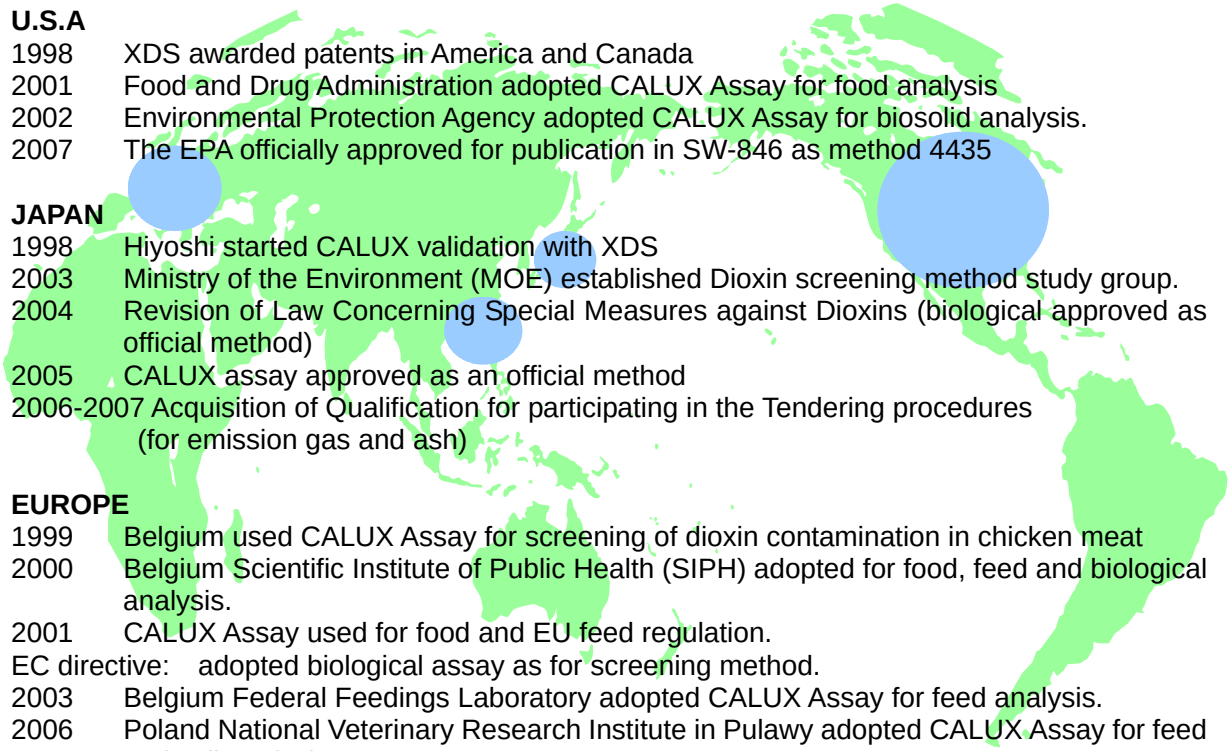
Usage of CALUX

We receive over 2,000 samples each year. Following are some example of usage of CALUX® Assay

- Monitoring of waste water, exhaust gas, and ash
- Monitoring of soil and sediment
- Initial screening for development of dioxin inhibitor or decomposer
- Epidemical research on biological sample
- Monitoring of foods and food products
- Ah-R activity Screening for chemical substances

WORLD CALUX

Since 1998, CALUX® is being used as widely as in U.S.A, Europe, and in Asia including Japan.



U.S.A

1998 XDS awarded patents in America and Canada
2001 Food and Drug Administration adopted CALUX Assay for food analysis
2002 Environmental Protection Agency adopted CALUX Assay for biosolid analysis.
2007 The EPA officially approved for publication in SW-846 as method 4435

JAPAN

1998 Hiyoshi started CALUX validation with XDS
2003 Ministry of the Environment (MOE) established Dioxin screening method study group.
2004 Revision of Law Concerning Special Measures against Dioxins (biological approved as official method)
2005 CALUX assay approved as an official method
2006-2007 Acquisition of Qualification for participating in the Tendering procedures (for emission gas and ash)

EUROPE

1999 Belgium used CALUX Assay for screening of dioxin contamination in chicken meat
2000 Belgium Scientific Institute of Public Health (SIPH) adopted for food, feed and biological analysis.
2001 CALUX Assay used for food and EU feed regulation.
EC directive: adopted biological assay as for screening method.
2003 Belgium Federal Feedings Laboratory adopted CALUX Assay for feed analysis.
2006 Poland National Veterinary Research Institute in Pulawy adopted CALUX Assay for feed and soil analysis

ASIA

2005 Taiwan Cheng-Shiu University (Super Micro Mass Research and Technology Center) adopted CALUX Assay for food analysis
2006- Internship and joint study with Tsinghua University, China.
Start internship program for Indian Student

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