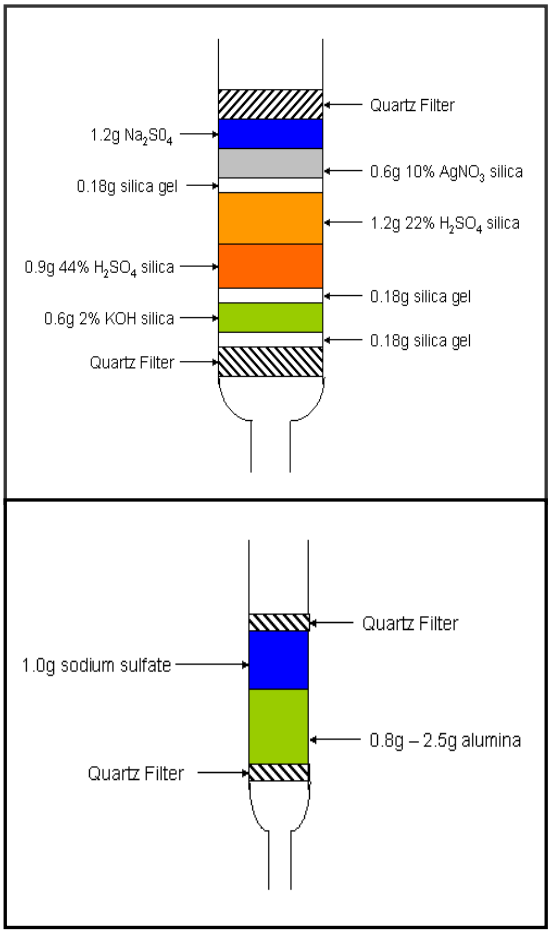
Procept™
Rapid Dioxin Assay

Application Note #501
Dioxin in Incinerator Ash

In 2004, the Japanese Ministry of the Environment reported on an evaluation they conducted comparing 11 different bioassay methods for measuring dioxins in fly ash, bottom ash and stack gases from garbage incinerators in Japan. The Procept technology, commercialized in Japan by Daiichi Fine Chemical Company as the AhRC PCR test (Aryl hydrocarbon Receptor Capture Polymerase Chain Reaction), was included in this study. This application note summarizes the performance of the AhRC PCR test in this evaluation study.

The sample preparation used in this study was a streamlined version of the traditional technique: soxhlet extraction followed by silica and alumina column clean up.

The sample was extracted twice at 150ºC, 1500-2000 psi into toluene containing 5% acetic acid using an ASE200 (Dionex.) Heating time was 7 minutes and static time was 15 minutes. Sample size was 1 gram for fly ash and 10 grams for bottom ash. The solvent was changed to hexane (10 mL) and the sample extract was run through a hexane washed multilayer silica column constructed as shown in the top figure to the right. The load solution was combined with the dioxin eluting fraction of 50 mL hexane.



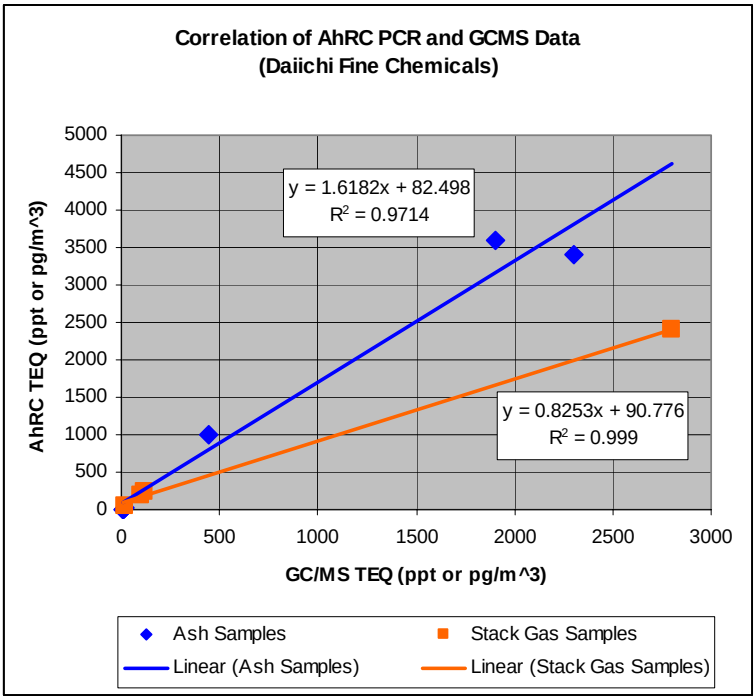
The combined 60mL of hexane was loaded onto a hexane washed alumina column constructed as shown in bottom figure to the right.

The alumina column was washed with successive 10mL fractions of:

hexane,
2% (v/v) dichloromethane/hexane; and
5% (v/v) dichloromethane/hexane (coplanar PCB fraction).

The dioxin fraction was eluted with 15 mL 50% (v/v) dichloromethane/hexane.

The dioxin and co-planar PCB fractions were evaporated and converted to 100 µL DMSO (or methanol). Five microliters of this solution was transferred to a glass vial in a 96 well rack containing 50 µL of assay buffer. Fifty µL of thawed activation solution was added and the rack wass placed on an orbital shaker for 1 hour. A capture strip was placed into a rack and prepared according the kit directions, and 30 µL of the solution in the glass vial was transferred to it. The rack containing the Capture Strip was placed on the orbital shaker for 30 minutes. After shaking, the Capture Strip was washed and PCR reagents were added (40 µL.) The strip was covered with optically clear film and placed in the PCR instrument for real time DNA amplification.



A number of fly ash, bottom ash and stack gas samples were analyzed and results were published in 2004. The figure to the left presents the correlation of the AhRC PCR (Procept Technology) data generated by Daiichi with the HR GCMS data generated by the Ministry of the Environment. This graph shows a linear correlation for each sample type (ash and stack gas) between the Procept Assay and HR GCMS results. The fly ash and bottom ash sample result correlation is linear (R^2 = 0.971) over a range from <10 ppt to > 2000 ppt. The stack gas correlation is linear (R^2 = 0.999) from 15 – 2500 pg/m^3.)

Other findings of the performance of this methodology reported in the paper:

Limit of Quantitation, *ash samples*:	**0.12 – 2.1 ppt**
Limit of Quantitation, *stack gas samples:*	**1.5 – 2.5 pg/m^3**
Reproducibility, *ash samples, CV (%):*	**1.3 – 6.2%**
Reproducibility, *stack gas samples, CV (%):*	**4.1 – 12%**

Conclusions:

The Japanese Ministry of the Environment concluded that this method, based on the Procept Dioxin Assay technology, is ***"already at applicable levels as [a] supplementary method for the standard GC/MS procedures."***

Reference:

S. Ota, M. Morita, S. Sakai, and K. Sudo, *Organohalogen Compounds*, **66**, 682(2004).

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