Importance of Dioxin/Furan Determination

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) comprise a group of ubiquitous environmental contaminants which pose a threat to humans and other organisms. These compounds are highly toxic and have been linked to cancer, liver damage, and various reproductive and developmental diseases.

There are 75 PCDD congeners and 135 PCDF congeners, each having its own chemical and toxic characteristics. The most widely known congener is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) which is also the most toxic. Toxic equivalency factors (TEFs) are used to represent the fraction of TCDD toxicity exhibited by a congener. A Toxicity Equivalence (TEQ) is derived from the concentration of each of the toxic congeners in the mixture.

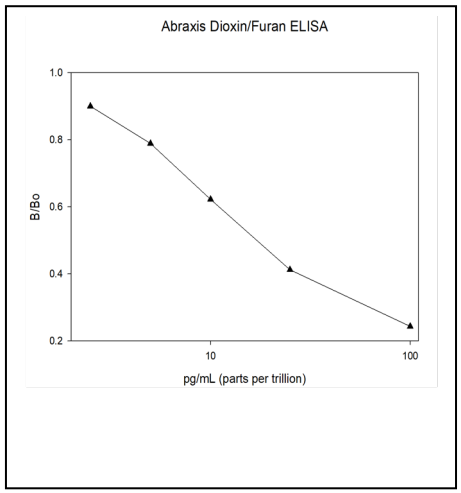
The analysis of PCDDs and PCDFs is of importance for environmental monitoring and human exposure assessment. The analysis of these compounds is very complex and expensive, limiting the number of samples that can be analyzed in a timely and cost-effective manner. GC-HRMS is the reference analytical method, requiring extensive sample clean-up and expensive instrumentation. Immunochemical methods, such as the ABRAXIS® Dioxin/Furan ELISA provides improvement in cost, sensitivity and sample throughput.

Performance Data

Assay range: The assay range for this assay is 2.5-50 pg/mL (ppt)

Test reproducibility: The standard curve R^2 must be ≥ 0.98. The absorbance Coefficient of variation (CVs) for standards should be ≤10% and for the samples should be ≤15%. The Standard 0 absorbance value should be between 0.8 - 3.000.

*Selectivity: The assay exhibits very good cross-reactivity with toxic congeners tested (cross-reactivity table is shown below)



Compound	Cross-Reactivity (%)	TEF
Dioxin Toxic Congeners		
2,3,7,8-TCDD	100	1
1,2,3,7,8-PnCDD	70	1
1,2,3,4,7,8-HxCDD	0.6	0.1
1,2,3,6,7,8-HxCDD	1.0	0.1
1,2,3,7,8,9-HxCDD	0.5	0.1
1,2,3,4,6,7,8-HpDD		0.01
OCDD	< 0.06	0.001
Furan Toxic Congeners		
2,3,7,8-TCDF	6	0.1
1,2,3,7,8-PnCDF	1.2	0.05
2,3,4,7,8-PnCDF	1.5	0.5
1,2,3,4,7,8-HxCDF	<0.06	0.1
1,2,3,7,8,9-HxCDF		0.1
2,3,4,6,7,8-HxCDF		0.1
1,2,3,4,6,7,8-HpCDF	<0.06	0.01
1,2,3,4,7,8,9-HpCDF		0.01
OCDF	<0.06	0.0001
PCB		
Aroclor 1221	<0.01	
Aroclor 1242	<0.1	
Aroclor 1254	<0.01	
Aroclor 1260	<0.01	

General Limited Warranty: Gold Standard Diagnostics warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Gold Standard Diagnostics makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

For ordering or technical assistance contact:
Gold Standard Diagnostics
795 Horsham Road
Horsham, PA 19044
Web: www.abraxiskits.com

Tel.: (215) 357-3911
Ordering: info.abraxis@us.goldstandarddiagnostics.com
Technical Support: support.abraxis@us.goldstandarddiagnostics.com

Date these instructions are effective : 03/18/2025

Version: 03

ABRAXIS® Dioxin/Furan ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Dioxin/Furan in Water, Soil and Sediment Samples

Product No. 530037

1. General Description

The ABRAXIS® Dioxin/Furan ELISA is an indirect immunoassay (ELISA) for the quantitative and sensitive detection of Dioxins and Furans in water, soil and sediment samples. If necessary, positive samples can be confirmed by instrumental analysis or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Dioxin. The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The ABRAXIS® Dioxin/Furan ELISA should be stored in the refrigerator (2-8°C). Solutions should be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the last day of the month as indicated by the expiration date on the box. Consult state, local and federal regulations for proper disposal of all reagents.

4. Test Principle

The test is an indirect competitive ELISA that allows the sensitive detection of Dioxins and Furans. It is based on the recognition of Dioxins and Furans congeners by specific antibodies. Dioxins and Furans when present in a sample and a Dioxin/Furan-protein analogue immobilized on the plate compete for the binding sites of antibodies in solution. After a washing step, a second antibody-HRP label is added. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of the Dioxin/Furan present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader.

5. Limitations of the ABRAXIS® Dioxin/Furan ELISA, Possible Test Interference

The ABRAXIS® Dioxin/Furan assay will detect many of the Dioxins (PCDDs) and Furans (PCDFs) that have a high TEF, please refer to the specificity table for data on the various congeners.

Numerous organic and inorganic compounds commonly found in water, soil and sediment samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in a give sample, test interferences caused by matrix effects can't be completely excluded.

Mistakes in handling the test can also cause errors. Possible sources for such errors can be: inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

As with any analytical technique (GC, HPLC, etc.) positive results requiring some action should be confirmed by an alternative method.

6. Working Instructions

A. Materials Provided

1. Microtiter plate coated with an analog of Dioxin/Furan conjugated to a protein
2. Standards (6): 0, 2.5, 5, 10, 25, 50 ppt, 1 mL each
3. Antibody solution, 7.5 mL
4. Anti-Rabbit-HRP Conjugate, 12 mL
5. ABRAXIS® Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section D)
6. Substrate (Color) Solution (TMB), 12 mL
7. Stop Solution, 12 mL
8. Diluent: 50% DMSO/water, 0.01% Triton X-100, 25 mL (For dilution of samples above the range of the curve)

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (50-250 µL)
2. Axygen® 300 µL Maxymum Recovery® universal fit pipette tips, Axygen Scientific product# T-350-C-L-R (highly recommended for pipetting standards, samples, and standards/samples mixed with antibody)
3. Multi-channel pipette (50-250 µL), stepper pipette (50-250 µL), or electronic repeating pipette with disposable plastic tips
4. Glass test tubes with holder
5. Deionized or distilled water
6. Container with 500 mL capacity (for diluted 1X Wash Buffer, see Test Preparation, Section D)
7. Graduated cylinder
8. Paper towels or equivalent absorbent material
9. Timer
10. Tape or parafilm
11. Microtiter plate washer (optional)
12. Microtiter plate reader (wavelength 450 nm)
13. Shaker for microtiter plates (optional)

C. Notes and Precautions

Micro-pipetting equipment and pipette tips (Axygen® 300 µL Maxymum Recovery® highly recommended) for pipetting the standards, samples, and standards/samples mixed with antibody are necessary.

We recommend using a multi-channel, stepping, or electronic repeating pipette for the addition of the antibody, enzyme conjugate, substrate (color), and stop solutions in order to equalize the incubation periods of the standard solutions and the samples on the entire microtiter plate.

To avoid drift and obtain accurate results, the addition of the antibody, standards/samples mixed with antibody, conjugate, substrate (color), and stop solutions should be performed in less than 2 minutes for each reagent. If additions to the entire microtiter plate cannot be completed in less than 2 minutes, run size should be decreased to the number of rows which can be pipetted in less than 2 minutes.

Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination. Read and understand the instructions and precautions given in this insert before proceeding.

D. Test Preparation

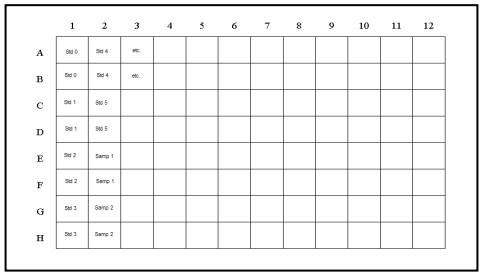
1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (2-8°C).
3. The standard, control, antibody solution, enzyme conjugate, substrate (color) and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the ABRAXIS® Wash Buffer (5X) concentrate at a ratio of 1:5 with deionized or distilled water. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.
5. The stop solution must be handled with care as it contains diluted H_2SO_4.

E. Working Scheme

The microtiter plate consists of 12 strips of 8, which can be used individually for the test. The standards must be run with each test. Never use the values of standards, which have been determined in a test performed previously.

Std0-Std5: Standards

Samp 1, Samp 2, etc.: Samples



F. Assay Procedure

1. **Add 125 µL of the standard solutions or samples** into a single glass test tube for each.
2. **Add 125 µL of antibody solution** (a stepping or electronic repeating pipette is recommended) to each tube. Vortex and **incubate for 60 minutes**.
3. **Add 100 µL of the standards/samples mixed with antibody** (from step 2) into duplicate wells of the test strips according to the working scheme given. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 30 seconds. Be careful not to spill contents. **Incubate for 60 minutes at room temperature.**
4. After incubation, remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. **Wash the strips four times using the diluted wash buffer.** Please use at least a volume of 250 µL of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
5. **Add 100 µL of Anti-Rabbit-HRP conjugate** to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 30 seconds. Be careful not to spill contents. **Incubate the strips for 30 minutes at room temperature.**
6. After incubation, remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. **Wash the strips four times using the diluted wash buffer.** Please use at least a volume of 250 µL of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
7. **Add 100 µL of substrate (color) solution** to the wells using a multi- channel, stepping, or electronic repeating pipette. **Incubated the strips for 20 minutes at room temperature.** Protect the strips from sunlight.
8. **Add 100 µL of stop solution** to the wells in the same sequence as for the color solution using a multi- channel, stepping, or electronic repeating pipette.
9. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after addition of the stop solution.

G. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4-parameter (preferred) or Logit/Log. For a manual evaluation calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value of each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on a vertical linear (y) axis versus the corresponding Dioxin/Furan concentration on horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for the samples will then yield levels in ppt of Dioxin/Furan by interpolation using the standard curve. Results can also be determined by using a spreadsheet macro available from Gold Standard Diagnostics upon request.

The concentrations of the samples are determined using this standard curve run with each test. Samples showing a lower concentration of Dioxin/Furan than Standard 1 (2.5 ppt) should be reported as containing < 2.5 ppt of Dioxin/Furan. Samples showing a higher concentration than standard 5 (50 ppt) must be diluted and re-analyzed to obtain more accurate results.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the standards. Samples with lower absorbances than a standard will have concentrations of Dioxin/Furan greater than that standard. Samples which have higher absorbances than a standard will have concentrations of Dioxin/Furan less than that standard.

As with any analytical technique (GC, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.