B. Limit of Detection
The ABRAXIS® Triclosan Plate Assay has an estimated minimum detection concentration based on a 95% B/Bo of 0.015 parts per billion (ppb).

C. Sensitivity
The ABRAXIS® Triclosan Plate Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 20 ppt (0.020 ppb). Refer to appropriate application notes or procedures for sensitivity in specific sample matrices.

D. 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 and Control should be ≤15%. The Control should be within its acceptable range and Standard 0 absorbance value should be between 0.8 - 3.000.

E. Recovery
Four (4) groundwater samples were spiked with various levels of Triclosan and then assayed using the ABRAXIS® Triclosan Plate Assay. The following results were obtained:

Amount of Triclosan Added (ppb)	Recovery Mean (ppb)	Recovery S.D. (ppb)	Recovery %
0.5	0.467	0.029	93
1	1.122	0.079	112
2	2.146	0.082	107
Average			104

F. Specificity
The cross-reactivity of the ABRAXIS® Triclosan Plate Assay for various related and unrelated compounds can be expressed as the least detectable dose (LDD) which is estimated at 90% B/Bo, or as the dose required to displace 50% (50% B/Bo).

Compound	LDD (ppb)	50% B/Bo (ppb
Triclosan	0.020	0.250
Triclosan methyl	0.015	0.080
PBDE Congener 28	0.034	0.61
PBDE Congener 47	0.020	0.390
PBDE Congener 49	5.2	17.8
PBDE Congener 99	2.15	15.0
4'-OH-BDE-47	0.13	7.8
5-OH-BDE-47	0.15	5.6
6-OH-BDE-47	0.66	10.2
2,4,5-Tribromobiphenyl	>100	>100
2,4',5-Tribromobiphenyl	54	9,100
2,3,7,8-Tetrachloro-dibenzo-p-dioxin	>100	>100
T3	0.94	40
L-Thyroxine (T4)	340	700

The following compounds demonstrated no reactivity in the Triclosan Plate Assay at concentrations up to 1,000 ppb: Biphenyl, 2,4-D.

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ABRAXIS® Triclosan ELISA Microtiter Plate
Product No. 530114

1. General Description
For detection of Triclosan and Triclosan methyl. Please refer to the attached specific procedures for water (groundwater, surface water, well water, effluent), and soil. Application procedures for other sample matrices can be obtained from Gold Standard Diagnostics.

2. Storage and Stability
Store all reagents at 2-8°C. Do not freeze. 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.

3. Test Principle
The ABRAXIS® Triclosan Microtiter Plate Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of Triclosan. In the assay system, standards, controls, or samples are added, along with an antibody specific to Triclosan, to microtiter wells coated with Goat Anti-Rabbit Antibody and incubated for thirty (30) minutes. The Triclosan enzyme conjugate is then added. At this point, a competitive reaction occurs between the Triclosan, which may be in the sample, and the enzyme labeled Triclosan analog for the antibody binding sites on the microtiter well. The reaction is allowed to continue for thirty (30) minutes. After a washing step, the presence of Triclosan is detected by adding the "Color Solution," which contains the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme labeled Triclosan bound to the Triclosan antibody catalyzes the conversion of the substrate/chromogen mixture to a colored product. The color reaction is stopped and stabilized after a twenty (20) minute incubation period by the addition of diluted acid (stop solution). The color is then evaluated using an ELISA reader.
A dose response curve of absorbance vs. concentration is generated using results obtained from the standards. The concentration of Triclosan present in the control and samples is determined directly from this curve. Since the labeled Triclosan (conjugate) was in competition with the unlabeled Triclosan (sample) for the antibody sites, the intensity of the color developed is inversely proportional to the concentration of Triclosan present in the sample.

4. Limitations of the ABRAXIS® Triclosan ELISA
The ABRAXIS® Triclosan Plate Assay will detect Triclosan and related Triclosan methyl compounds. Refer to the specificity table for data on several related compounds. The ABRAXIS® Triclosan Plate Assay kit provides screening results. As with any analytical technique (GC, HPLC, etc...) positive results requiring some action should be confirmed by an alternative method.

5. Working Instructions
A. Materials Provided
1. Microtiter Plate (8 X 12 strips) coated with Goat-Anti Rabbit Antibody
2. Triclosan Antibody Solution, 6 mL
3. Triclosan Standards (7) (0, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5 ppb) 1.0 mL
4. Control (0.75 ± 0.15 ppb ppb) 1.0 mL
5. Triclosan Enzyme Conjugate 6 mL
6. Diluent/Zero Standard (Sample Diluent) 30 mL
7. Color Solution 12 mL

8. Stop Solution 6 mL
9. ABRAXIS® Wash Buffer (5x) Concentrate 100 mL

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

1. Precision pipets capable of delivering 50, 100 and 250 µL, and tips*
2. Tape or Parafilm®*
3. Timer*
4. Distilled or deionized water for diluting Wash Buffer
5. Storage bottle with 500 mL capacity for storage of 1X Wash Buffer
6. Microplate or strip reader capable of reading absorbance at 450 nm

C. Reagent Preparation

All reagents must be allowed to come to room temperature.

Dilute the wash buffer (5X) concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add 400 mL of deionized or distilled water. This solution is used to wash the antibody coated wells.

D. Sample Information

This procedure is recommended for use with water samples. Other samples may require modifications to the procedure and should be thoroughly validated.

**Water samples and controls should be collected in glass vessels (Teflon-lined caps). Immediately upon collection, methanol (HPLC grade) should be added to the samples (25% v/v final concentration) to prevent adsorptive losses to the glass containers.

Samples containing gross particulate matter should then be filtered (e.g. 0.2 um Anotop™ 25 Plus, Whatman, Inc.) to remove particles.

Samples that have been preserved with monochloroacetic acid or other acids, should be neutralized with strong base e.g. 6N NaOH, prior to assay.

If the Triclosan concentration of a sample exceeds 2.5 ppb, the sample is subject to repeat testing using a diluted sample. A ten-fold or greater dilution of the sample is recommended with an appropriate amount of Diluent/Zero Standard or Sample Diluent. For example, in a separate test tube make a ten-fold dilution by adding 100 µL of the sample to 900 µL of Diluent/Zero Standard. Mix thoroughly before assaying. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtain by the dilution factor, e.g. 10.

The presence of the following substances up to 1,000 ppm were found to have no significant effect on the Triclosan Plate Assay results: phosphate, sodium fluoride, sodium chloride, magnesium and humic acid. Nitrate, manganese, calcium, sodium thiosulfate and sulfate up to 10,000 ppm. Copper, iron and zinc up to 100 ppm.

E. Procedural Notes and Precautions

As with all immunoassays, a consistent technique is the key to optimal performance. To obtain the greatest precision, be sure to treat each well in an identical manner.

Add reagents directly to the bottom of the well while avoiding contact between the reagents and the pipet tip. This will help assure consistent quantities of reagent in the test mixture.

Avoid cross-contamination and carryover of reagents by using clean pipets for each sample addition and by avoiding contact between reagent droplets on the tubes and pipet tips.

The microtiter plate consists of 12 strips of 8 wells. If fewer than twelve strips are used, remove the unneeded strips and store refrigerated in the resealable foil bag (with desiccant) provided.

If more than 3 strips are being used per run, the use of a multi-channel pipette is recommended for the addition of conjugate, antibody, color, and stopping solutions.

Do not use any reagents beyond their stated shelf life. Each component used in any one assay should be of the same lot number and stored under identical conditions.

Avoid contact of Stop Solution (diluted sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

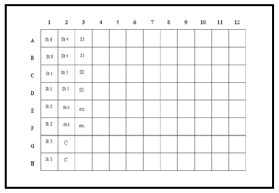
F. Quality Control

A control solution at approximately 0.75 ppb of Triclosan is provided with the ABRAXIS® Triclosan Plate Assay kit. It is recommended that it be included in every run and treated in the same manner as unknown samples. Acceptable limits should be established by each laboratory.

G. Working Scheme

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

St0-St6: Standards
C: Control
S1-Sx: Samples



H. Assay Procedure

1. **Add 50 µL of the appropriate standard, control, or sample**. Analysis in duplicates or triplicates is recommended.
2. **Add 50 µL of Triclosan antibody solution** successively to each well. Cover wells with parafilm or tape to prevent contamination and evaporation. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill the contents. **Incubate at ambient temperature for 30 minutes**.
3. After the incubation, **add 50 µL of Triclosan enzyme conjugate solution** successively to each well. Cover wells with parafilm or tape and thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. **Incubate at ambient temperature for 30 minutes.**
4. After the incubation, carefully remove the covering and vigorously shake the contents of the wells into a waste container. **Wash the strips three times with the diluted Wash Buffer** (see Reagent Preparation) by adding a volume of at least 250 µL of 1X Wash Buffer to each well and each washing step. Vigorously shake the contents of the wells into a waste container. Blot the inverted plate after each washing 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 Color Solution** successively to each well. Cover wells with parafilm or tape. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. **Incubate at ambient temperature for 20 minutes**.
6. **Add 50 µL of Stop Solution** successively to each well.
7. Read absorbance using a microplate reader at 450 nm within 15 minutes after adding the Stopping Solution.

I. Results

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-parameter or alternatively point to point). For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/Bo for each standard by dividing the mean absorbance value for each standard by the mean absorbance value for the Diluent/Zero Standard (Standard 0). Construct a standard curve by plotting the %B/Bo for each standard on the vertical linear (Y) axis versus the corresponding Triclosan concentration on the horizontal log (X) axis graph paper provided. Calculate the %B/Bo for the control and sample(s) and obtain the concentration of Triclosan (in ppb) by interpolation using the constructed standard curve.

**Multiply the sample and control results by a factor of 1.33 to account for the initial dilution of sample with methanol. Alternatively, program the microplate reader to automatically correct for the dilution factor.

Samples exhibiting a concentration lower than 0.015 ppb should be assumed to be below the detection limit of the assay. Samples exhibiting a concentration higher than 2.5 ppb must be diluted to obtain accurate results

6. Performance Data

A. Precision

The following results were obtained:

Control	1	2	3
Replicates	5	5	5
Days	5	5	5
n	25	25	25
Mean (ppb)	0.097	0.236	0.926
% CV (within assay)	9.7	7.3	8.3
% CV (between assay)	12.4	12.4	9.6