## B. Limit of Detection

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

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

## D. Recovery

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

| Amount of | Recovery | | |
|---|---|---|---|
| Sulfamethoxazole Added (ppb) | Mean (ppb) | S.D. (ppb) | % |
| 0.05 | 0.05 | 0.010 | 96 |
| 0.10 | 0.09 | 0.009 | 92 |
| 0.25 | 0.25 | 0.015 | 99 |
| 0.50 | 0.50 | 0.031 | 100 |
| Average | | | 97 |

## D. Specificity

The cross-reactivity of the ABRAXIS® Sulfamethoxazole Plate Assay for various related compounds can be expressed as the least detectable dose (LDD) which is estimated at 95% B/Bo, or as the dose required for 50% absorbance inhibition (50% B/Bo).

| Compound | LDD (ppb) | 50% B/Bo (ppb) |
|---|---|---|
| Sulfamethoxazole | 0.015 | 0.255 |
| Sulfamethoxypyridazine | 0.020 | 0.146 |
| Sulfachloropyridazine | 0.019 | 0.180 |
| Sulfadimethoxine | 0.016 | 0.420 |
| Sulfamethizole | 0.116 | 2.50 |
| Sulfasalazine | 0.450 | 7.90 |
| Sulfapyridine | 0.365 | 7.60 |
| Sulfameter | 0.068 | 12.0 |
| Sulfaquinoxaline Sodium Salt | 0.130 | 26.5 |
| Sulfadiazine | 6.80 | 120 |
| Sulfacetamide Sodium Salt | 31.0 | 250 |
| Sulfamerazine | 11.8 | 580 |
| Sulfaguanidine | 51.0 | 1010 |
| Sulfabenzamide | 73.0 | 1750 |
| Sulfamethazine | 135 | 7600 |

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Version: 03

# ABRAXIS® Sulfamethoxazole ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Sulfamethoxazole and related Sulfa compounds in water

**Product No. 522003**

## 1. General Description

The ABRAXIS® Sulfamethoxazole Plate Assay will detect Sulfamethoxazole and related Sulfa compounds in water (groundwater, surface water, well water). For soil, crop, and food use contact the Gold Standard Diagnostics for application bulletins and/or specific matrix validation guidelines. Refer to the specificity table for data on several related compounds. The Sulfamethoxazole 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.

## 2. Safety Instructions

Discard samples according to local, state, and federal regulations

## 3. Reagent 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.

## 4. Principle

The ABRAXIS® Sulfamethoxazole Microtiter Plate Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of Sulfamethoxazole. In the assay system, standards, controls, or samples are added, along with an antibody specific to Sulfamethoxazole, to microtiter wells coated with Goat Anti-Rabbit Antibody and incubated for twenty (20) minutes. The Sulfamethoxazole enzyme conjugate is then added. At this point, a competitive reaction occurs between the Sulfamethoxazole, which may be in the sample, and the enzyme-labeled Sulfamethoxazole analog for the antibody binding sites on the microtiter well. The reaction is allowed to continue for forty (40) minutes. After a washing step, the presence of Sulfamethoxazole 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 Sulfamethoxazole bound to the Sulfamethoxazole antibody catalyzes the conversion of the substrate/chromogen mixture to a colored product. The color reaction is stopped and stabilized after a thirty (30) minute incubation period by the addition of diluted acid (stopping 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 Sulfamethoxazole present in the control and samples is determined directly from this curve. Since the labeled Sulfamethoxazole (conjugate) was in competition with the unlabeled Sulfamethoxazole (sample) for the antibody sites, t**he intensity of the color developed is inversely proportional to the concentration of Sulfamethoxazole present in the sample.**

## 5. Limitations of the Sulfamethoxazole ELISA, Possible Test Interference

Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded.

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

Each reagent is optimized for use in the ABRAXIS® Sulfamethoxazole ELISA kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other ABRAXIS® Sulfamethoxazole ELISA Kits with different lot numbers. As with any analytical technique (GC/MS, HPLC, etc.), samples requiring regulatory action should be confirmed by an alternative method.

## 6. Working Instructions

### A. Materials Provided

1. Microtiter Plate coated with Goat-Anti Rabbit Antibody
2. Sulfamethoxazole antibody (rabbit anti-Sulfamethoxazole); 6 mL
3. Sulfamethoxazole Enzyme Conjugate; 6 mL
4. Sulfamethoxazole Standards (6); 0, 0.025, 0.05, 0.1, 0.25, 1.0 ppb; 1.0 mL
5. Control (1) 0.2 ppb ± 0.04 ppb; 1.0 mL
6. Diluent/Zero Standard (Sample Diluent); 30 mL
7. Color Solution; 16 mL
8. Stop Solution; 12 mL
9. ABRAXIS® Washing Buffer (5X) Concentrate; 100 mL

### B. Materials Required but Not Provided (not delivered with the test kit)

1. Precision pipets capable of delivering 50, 75, 100, 150, and 250 uL, and tips*
2. Tape or Parafilm®*
3. Timer*
4. Distilled or deionized water for diluting Wash Buffer
5. Storage bottle with 1000 mL capacity for storage of 1x Wash Buffer*
6. Microplate or strip reader capable of reading absorbance at 450 nm*

* Please contact Gold Standard Diagnostics for supplier information.

### C. Sample Information

This procedure is recommended for use with water samples. Other samples may require modifications to the procedure and should be thoroughly validated. Samples containing gross particulate matter should be filtered (e.g. 0.2 um Anotop™ 25 Plus, Whatman, Inc.) to remove particles. Samples which have been preserved with monochloroacetic acid or other acids, should be neutralized with strong base e.g. 6N NaOH, prior to assay. If the Sulfamethoxazole concentration of a sample exceeds 1.0 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 uL of the sample to 900 uL 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 10,000 ppm were found to have no significant effect on the Sulfamethoxazole Plate Assay results: sulfate, phosphate, magnesium, and calcium. Nitrate, sodium chloride, sodium fluoride, manganese, and zinc up to 1,000 ppm. Copper and iron up to 100 ppm. Humic Acid up to 10 ppm. Sodium Thiosulfate up to 1 ppm.

### D. Reagent Preparation

All reagents must be allowed to come to room temperature.

**ABRAXIS® Wash Buffer (5X)**

In a 1000 mL container, dilute the wash buffer concentrate 1:5 by the addition of distilled or deionized water (i.e., 100 mL of wash buffer concentrate plus 400 mL of $H_2O$). This solution is used to wash the antibody coated wells.

### 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-contaminations 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 Stopping Solution (diluted sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

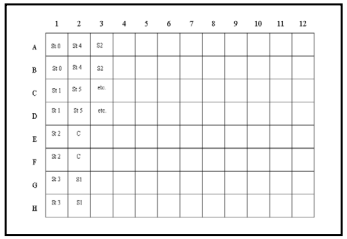
### F. Quality Control

A control solution at approximately 0.2 ppb of Sulfamethoxazole is provided with the ABRAXIS® Sulfamethoxazole 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. Assay Procedure

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

St0-St5: Standards
C: Control
S1-Sx: Samples




1. **Add 75 uL of the appropriate standard, control, or sample.** Analysis in duplicates or triplicates is recommended.
2. **Add 50 uL of Sulfamethoxazole 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 20 minutes.
3. After the incubation, **add 50 uL of Sulfamethoxazole 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 40 minutes**.
4. After the incubation, carefully remove the covering and vigorously shake the contents of the wells into a waste container. **Wash the strips with the diluted Wash Buffer** (see Reagent Preparation) by adding a volume of at least 250 uL of Wash Buffer to each well. Vigorously shake the contents of the wells into a waste container. Any remaining buffer in the wells should be removed by patting the plate on a dry stack of paper towels. Repeat this wash step two times, for a total of 3 rinses.
7. **Add 150 uL 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 30 minutes.
8. **Add 100 uL of Stopping Solution** successively to each well.
9. Read absorbance using a microplate reader at 450 nm within 15 minutes after adding the Stopping Solution.

### H. Evaluation

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 Sulfamethoxazole concentration on the horizontal log (X) axis on the graph paper provided. Calculate the %B/Bo for the control and sample(s) and obtain the concentration of Sulfamethoxazole (in ppb) by interpolation using the constructed standard curve.

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 1.0 ppb must be diluted to obtain accurate results.

## 7. Performance Data

### A. Precision

The following results were obtained:

| Control | 1 | 2 | 3 |
|---|---|---|---|
| Replicates | 3 | 3 | 3 |
| Days | 5 | 5 | 5 |
| n | 15 | 15 | 15 |
| Mean (ppb) | 0.056 | 0.208 | 0.499 |
| % CV (within assay) | 12.5 | 8.6 | 2.8 |
| % CV (between assay) | 13.2 | 4.6 | 3.7 |