

Sulfamethazine

• Intended Use

For the detection and quantitation of sulfamethazine in water (groundwater, surface water, well water). For other use contact the company for application bulletins and/or specific matrix validation guidelines.

• Principle

The Abraxis Sulfamethazine Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of sulfamethazine. In the assay system, standards or samples to be tested are added, along with an enzyme conjugate, and an antibody specific for sulfamethazine, to microtiter wells coated with Goat anti-rabbit antibody. At this point a competitive reaction occurs between the sulfamethazine which may be in the sample and the enzyme labeled sulfamethazine for the antibody binding sites. The reaction is allowed to incubate for sixty (60) minutes. At the end of the incubation period, the wells are washed with Washing Buffer.

The presence of sulfamethazine 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 sulfamethazine bound to the sulfamethazine antibody catalyzes the conversion of the substrate/ chromogen mixture to a colored product. After a thirty (30) minute incubation period, the reaction is stopped and stabilized by the addition of a diluted acid (Stopping Solution). Since the labeled sulfamethazine (conjugate) was in competition with the unlabeled sulfamethazine (sample) for the antibody sites, the **color developed is inversely proportional to the concentration of sulfamethazine in the sample.**

• Reagents

The Abraxis Sulfamethazine Plate Kit contains the following items:

- 1. Microtiter Plate coated with Goat-Anti Rabbit Antibody**
96 test kit: 12 strips of 8 antibody coated wells and strip holder (1).
- 2. Sulfamethazine Antibody Solution**
Sulfamethazine antibody (rabbit anti-Sulfamethazine) solution in a buffered saline solution with preservative and stabilizers.
96 test kit: One vial containing 6 mL
- 3. Sulfamethazine Enzyme Conjugate**
Horseradish peroxidase (HRP) labeled Sulfamethazine analog in a buffered solution with preservative and stabilizers.
96 test kit: One vial containing 6 mL
- 4. Sulfamethazine Standards**
Four concentrations (0, 0.05, 0.15, 0.25, 0.50, 1.5, 5.0 ppb) of Sulfamethazine standards in distilled water with preservative and stabilizers.
96 test kit: Each vial contains 1.0 mL
- 5. Diluent/Zero Standard (Sample Diluent)**
Distilled water with preservative and stabilizers without any detectable Sulfamethazine.
96 test kit: One bottle containing 30 mL
- 6. Color Solution**
A solution of hydrogen peroxide and 3,3',5,5'-tetramethyl benzidine in an organic base.
96 test kit: One bottle containing 12 mL
- 7. Stopping Solution**
A solution of diluted acid.
96 test kit: Two bottles containing 6 mL each
- 8. Washing Buffer (5x) Concentrate**
Buffered salts with detergent and preservatives.
96 test kit: One bottle containing 100 mL

• Reagent Storage and Stability

Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the expiration date on the box.

Consult state, local and federal regulations for proper disposal of all reagents.

• Materials Required but Not Provided

In addition to the reagents provided, the following items are essential for the performance of the test:

Precision pipets capable of delivering 50, 75, 100, 150, and 250 μ L, and tips*

Tape or Parafilm®*

Timer*

Distilled or deionized water for diluting Wash Buffer

Storage bottle with 1000 mL capacity for storage of 1x Wash Buffer*

Microplate or strip reader capable of reading absorbance at 450 nm*

* Please contact Abraxis for supplier information.

• 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 μ m 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 Sulfamethazine concentration of a sample exceeds 5.0 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 μ L of the sample to 900 μ L 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.

• Reagent Preparation

All reagents must be allowed to come to room temperature.

Wash Buffer

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 H₂O). This solution is used to wash the antibody coated wells.

• 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.

Do not use any reagents beyond their stated shelf life.

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

• Limitations

The Abraxis Sulfamethazine Assay will detect sulfamethazine and other sulfonamides to different degrees. Refer to specificity table for data on several of the sulfonamides. The Abraxis Sulfamethazine 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.

• Assay Procedure

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

St0-St6: Standards
S1-Sx: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	St 0	St 4	St 2									
B	St 0	St 4	St 2									
C	St 1	St 5	St 3									
D	St 1	St 5	St 3									
E	St 1	St 5										
F	St 1	St 5										
G	St 1	St 5										
H	St 1	St 5										

1. Add 50 μ L of the appropriate standard or sample. Analysis in duplicates or triplicates is recommended.
2. Add 50 μ L of Sulfamethazine Enzyme Conjugate.
3. Add 50 μ L of Sulfamethazine 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 60 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 μ L 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.

- Add 100 μ L 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.
- Add 50 μ L of Stopping Solution successively to each well.
- Read absorbance using a microplate reader at 450 nm within 15 minutes after adding the Stopping Solution.

• 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 Sulfamethazine 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 Sulfamethazine (in ppb) by interpolation using the constructed standard curve.

Samples exhibiting a concentration lower than 0.05 ppb should be assumed to be below the detection limit of the assay. Samples exhibiting a concentration higher than 5.0 ppb must be diluted to obtain accurate results.

• Expected Results

In a study with water samples from locations across the U.S., the Abraxis Sulfamethazine Assay was shown to correlate well with another commercial Sulfamethazine immunoassay.

• Performance Data

Precision

The following results were obtained:

Control	0.15	0.50	1.5
Replicates	5	5	5
Days	5	5	5
n	25	25	25
Mean (ppb)	0.156	0.52	1.37
% CV (within assay)	10.8	10.4	8.7
% CV (between assay)	15.6	11.8	10.9

Sensitivity

The Abraxis Sulfamethazine Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 0.04 ppb.

Recovery

Five (5) groundwater samples, were spiked with various levels of sulfamethazine and then assayed using the Abraxis Sulfamethazine Assay. The following results were obtained:

Amount of Sulfamethazine Added (ppb)	Recovery		
	Mean (ppb)	S.D. (ppb)	%
0.25	0.29	0.05	117
1.0	0.94	0.05	94
2.5	1.99	0.08	80
Average			97

Specificity

The cross-reactivity of the Abraxis Sulfamethazine Assay for various sulfonamides can be expressed as the least detectable dose (LDD) which is estimated at 90% B/Bo, or as the dose required for 50% absorbance inhibition (50% B/Bo).

B/Bo Compound	50% (ppb)	Cross-Reactivity (%)
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Sulfamethazine	0.6	100
Sulfacetamide	0.6	100
Sulfamerazine	4.0	15
Sulfathiazole	4.1	15

The following sulfa drugs gave cross-reactivity of < 1%: sulfasalazine, sulfasomidin, sulfatran, sulfisoxazole, sulfiphenazole, sulfaguanadine, sulfachloropyridazine, sulfaquinoxaline, sulfadiazine, sulfamethoxyppyridazine, sulfameter, sulfadimethoxine, sulfamilamide, sulfapyridine, sulfabenzamide, sulfamethoxazole.

The following compounds demonstrated no reactivity in the Abraxis Sulfamethazine Assay at concentrations up to 1000 ppb: aldicarb, aldicarb sulfoxide, aldicarb sulfone, alachlor, benomyl, butachlor, butylate, captan, carbaryl, carbendazim, carbofuran, 2,4-D, 1,3-dichloropropene, dinoseb, MCPA, metolachlor, metribuzin, pentachlorophenol, picloram, propachlor, terbufos, thiabendazole, and thiophanate-methyl.

• Ordering information

Abraxis Sulfamethazine Assay Kit 96T PN 515006

• Assistance

For ordering or technical assistance contact:

Abraxis LLC
54 Steamwhistle Drive
Warminster, Pennsylvania, 18974

Phone: (215) 357-3911 * Fax: (215) 357-5232

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• General Limited Warranty

Abraxis LLC 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. Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.

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