

## Importance of Streptomycin Determination

Antibiotic residues in foods and water pose a serious threat to public health. Streptomycin is a broad spectrum Aminoglycoside antibiotic used in humans for the treatment of moderate to severe bacterial infections. Treatment with Streptomycin must be monitored due to the risk of serious adverse effects, especially in those with impaired renal function. Nerve toxicity, including ototoxicity, which can damage both vestibular and cochlear function, optic nerve dysfunction, and peripheral neuritis may result. The use of Streptomycin in infants is limited, as it can impair the functioning of the central nervous system, reducing respiration, and may lead to coma. Streptomycin is also typically avoided in the treatment of pregnant women, due to the risk of ototoxicity to the fetus (resulting in deafness), and in women who are nursing, as it is excreted in bodily fluids including breast milk, saliva, and sweat. Streptomycin is also used in many food producing animals, including beef and dairy cattle, sheep, pigs, and poultry, as well as in honeybees. The potential for harmful effects on human health has led to restrictions in its use in food producing animals in many countries including the United States, Australia and the European Union. In the U.S., Streptomycin residues cannot exceed 2.0 ppm in chicken, pig, or cattle kidney and 0.5 ppm in other tissues. In Australia, residues cannot exceed 0.3 ppm in meat and 0.2 ppm in milk. In the E.U., residues cannot exceed 0.5 ppm in meat and 0.2 ppm in milk. Honey produced or imported into the E.U. can have no detectable levels of any antibiotic. Contamination in honey can occur not only through direct treatment of the bees but also through the extensive use of Streptomycin as a pesticide in a variety of plants. Due to its use in the control of fireblight on apple and pear trees, an estimated 20% of apples and 30% of pears produced in the U.S. contain Streptomycin residues at 0.25 ppm (the regulatory limit in the U.S. and Canada). Vegetable plants, including celery, tomato, pepper, and potato plants as well as ornamental plants such as philodendron, chrysanthemums, and roses and a variety of plant seeds are also commonly treated with Streptomycin. An effective algacide, Streptomycin is also used in ponds and aquariums. The monitoring of water sources, including agricultural run-off, and food products, including meat, milk, and produce, for antibiotic residues is necessary to ascertain that these compounds are not misused and do not present a danger to human and animal health.

The Abraxis Streptomycin ELISA allows the determination of 41 samples in duplicate determination. Only a few grams or milliliters of sample are required. A sample preparation may be necessary prior to assaying (see section H. Preparation of Samples). The ELISA analysis can be performed in less than 1 hour.

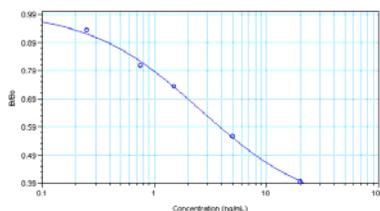
## Performance Data

Test sensitivity: The sensitivities in various matrices are as follows:

Honey	5 ppb
Raw Milk	12.5 ppb

Standard Curve: Determinations closer to the middle of the calibration range of the test yield the most accurate results.

These values are used for demonstration purposes only; do not use these values for your determinations:



Test reproducibility: Coefficients of variation (CVs) for standards: <10%; CVs for samples: <15%.

Selectivity: This ELISA recognizes Streptomycin and related compounds with varying degrees:

Cross-reactivities:	Streptomycin	100%
	Dihydrostreptomycin	120%

Samples: To eliminate matrix effects in milk or honey samples, a sample clean-up is required (see section H. Preparation of Samples).

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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## Streptomycin ELISA, Microtiter Plate

### Enzyme-Linked Immunosorbent Assay for the Determination of Streptomycin in Contaminated Samples

Product No. 5111SA

#### 1. General Description

The Streptomycin ELISA is an immunoassay for the detection of Streptomycin. This test is suitable for the quantitative and/or qualitative detection of Streptomycin in contaminated samples. Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

#### 2. Safety Instructions

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

#### 3. Storage and Stability

The Streptomycin ELISA Kit should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20–25°C) before use. Reagents may be used until the expiration date on the box.

#### 4. Test Principle

The Abraxis Streptomycin Plate Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of Streptomycin. Calibrators and samples (please refer to Sample Preparation section) are added, along with a Streptomycin-HRP enzyme conjugate, to wells in a microtiter plate that contain immobilized antibodies specific for Streptomycin. At this point, a competitive reaction occurs between the Streptomycin which may be in the sample and the enzyme labeled Streptomycin for the binding sites of the antibodies on the microtiter wells. The reaction is allowed to continue for thirty (30) minutes. After a washing step, the presence of Streptomycin is detected by adding the substrate ("Color Solution"), which contains the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled Streptomycin bound to the Streptomycin 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) and the color is evaluated using an ELISA plate reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run. Since the labeled Streptomycin (conjugate) was in competition with the unlabeled Streptomycin (sample) for the antibody sites, the color developed is inversely proportional to the concentration of Streptomycin in the sample.

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

Numerous organic and inorganic compounds commonly found in 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 samples, test interferences caused by matrix effects can not 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, 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 during the test performance (lower than 10°C or higher than 30°C).

The Abraxis Streptomycin ELISA Kit provides screening results. As with any analytical technique (GC, HPLC, etc.), samples requiring regulatory action should be confirmed by an alternative method.

#### Working Instructions

##### A. Materials Provided

1. Anti-Streptomycin Antibody Coated Microtiter Plate (12 X 8 strips) in a resealable foil pouch.
2. Streptomycin Standards/Calibrators (6): 0, 0.25, 0.75, 1.5, 5.0, and 20.0 ppb, 1.0 mL each.
3. Streptomycin-HRP Conjugate Solution, 11 mL.
4. Sample Diluent (10X) Concentrate, 2 X 25 mL.
5. Wash Solution (5X) Concentrate, 100 mL.
6. Color (Substrate) Solution (TMB), 12 mL.
7. Stop Solution, 12 mL.

## B. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the conjugate, substrate and stop solutions in order to equalize the incubation periods of the solutions on the entire microtiter plate. Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

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 (4-8°C).
3. The standard solutions, conjugate, substrate and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the **Sample Diluent Concentrate** at a ratio of 1:10. If using both bottles (50 mL total), add to 450 mL of deionized or distilled water.
5. Dilute the **Wash Buffer Concentrate** at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water.
6. The stop solution should be handled with care as it contains diluted H<sub>2</sub>SO<sub>4</sub>.

## C. Assay Procedure

1. Add 50 µL of the **standard solutions and samples** into the wells of the test strips according to the working scheme given (see section G). We recommend using duplicates or triplicates.
2. Add 100 µL of **enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Be careful not to spill contents.
3. Incubate the strips for 30 minutes at room temperature.
4. After incubation, remove the covering and vigorously shake the contents of the wells into a sink or other waste container. Wash the strips using the **diluted wash buffer solution** (see section B, step 5). Add a volume of 250 µL of wash buffer to each well. Vigorously shake the contents of the wells into the waste container. Any remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels. Repeat this wash step twice, for a **total of 3 rinses**.
5. Add 100 µL of **substrate (color) solution** to each well successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Be careful not to spill contents. Incubate the strips for 30 minutes at room temperature. Protect the strips from direct sunlight.
6. Add 100 µL of **stop solution** to each well in the same sequence as for the substrate solution using a multi-channel pipette or a stepping pipette.
7. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

## D. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log). For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/B<sub>0</sub> for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/B<sub>0</sub> for each standard on the vertical linear (y) axis versus the corresponding Streptomycin concentration on the horizontal logarithmic (x) axis on graph paper. %B/B<sub>0</sub> for samples will then yield levels of Streptomycin in ppb by interpolation using the constructed standard curve. Samples showing lower concentrations of Streptomycin compared to Standard 1 (0.25 ppb) should be reported as containing < 0.25 ppb. Samples showing a higher concentration than Standard 5 (20.0 ppb) must be diluted further to obtain accurate results.

## E. Additional Materials (not included with the test kit)

1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
2. Multi-channel pipette (10-250 µL) or stepper pipette with plastic tips (10-250 µL)
3. Microtiter plate reader (wavelength 450 nm)
4. Vortex Mixer

5. Glass tubes or vials with caps
6. Timer
7. Tape or Parafilm

## F. Additional Reagents (not included with the test kit)

1. Methanol, reagent grade

## G. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, 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.

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

Std 0-Std 6: Standards

0: 0.25; 0.75; 1.5; 5.0; 20.0 ppb

Samp1, Samp2, etc.: Samples

## H. Preparation of Samples

### Milk

1. Dilute Sample Diluent Concentrate as indicated in section B, step 4.
2. Add 1 mL of milk sample to a clean glass vial. Add 3 mL of diluted Sample Diluent to the vial. Vortex thoroughly.
3. Add 1.15 mL of diluted Sample Diluent to a second vial. Add 100 µL of the milk/diluent mixture (step 2). Vortex thoroughly. This will then be analyzed as sample (Assay Procedure, step 1).

The ELISA result must be multiplied by a factor of 50 to obtain the final Streptomycin concentration in the sample (the factor is necessary to account for the sample dilution required to remove matrix effects). Samples showing lower concentrations than standard 1 (0.25 ppb) should not be multiplied by the factor (50) but should be reported as containing <12.5 ppb. Highly contaminated samples (those outside of the calibration range of the assay) must be diluted and re-analyzed.

### Honey

1. Dilute Sample Diluent Concentrate as indicated in section B, step 4.
2. Add 1 mL of honey sample to a clean glass vial. Add 3 mL of diluted Sample Diluent to the vial. Vortex until honey is completely dissolved.
3. Add 320 µL of diluted Sample Diluent and 80 µL of methanol to a second vial. Vortex.
4. Add 100 µL of the honey/diluent mixture (step 2) to the diluent/methanol mixture in the second vial. Vortex thoroughly. This will then be analyzed as sample (Assay Procedure, step 1).

The ELISA result must be multiplied by a factor of 20 to obtain the final Streptomycin concentration in the sample (the factor is necessary to account for the sample dilution required to remove matrix effects). Samples showing lower concentrations than standard 1 (0.25 ppb) should not be multiplied by the factor (20) but should be reported as containing <5 ppb. Highly contaminated samples (those outside of the calibration range of the assay) must be diluted and re-analyzed.

For sample preparation procedures for additional matrices, please contact Abraxis LLC.