Importance of Gentamicin Determination

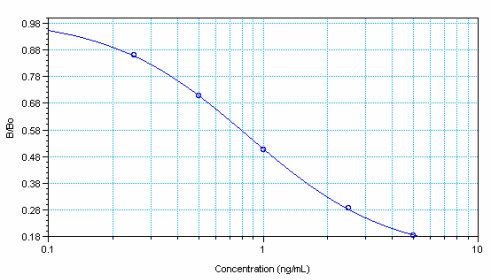
Antibiotic residues in foods pose a serious threat to public health. Gentamicin is an aminoglycoside antibiotic used for the treatment and prevention of infections caused by gram-negative and certain gram-positive bacteria including *Staphylococcus*. Treatment is administered intravenously, intramuscularly, or topically, as Gentamicin is not readily absorbed when administered orally. Gentamicin is not metabolized by the body and is released at various rates from different body tissues. Dosing must be carefully monitored, as Gentamicin has a narrow therapeutic range. Even at therapeutic levels, Gentamicin may be ototoxic, nephrotoxic, or cause nerve damage. Ototoxicity may cause damage to the inner ear, affecting balance, and can also result in hearing loss. Nephrotoxicity may cause cellular necrosis, which can result in acute renal failure.

The monitoring of water sources and food products, such as meat, milk and honey, for antibiotic residues is necessary to ascertain that these compounds are not misused and do not present a danger to human or animal health. In the European Union, the current maximum residue limit (MRL) for Gentamicin in milk is 100 µg/L and in meat is 50 µg/g.

The Abraxis Gentamicin ELISA allows the determination of 42 samples in duplicate determination. Only a few milliliters of sample are required. The test can be performed in less than 1 hour.

Performance Data

Test sensitivity: The limit of detection for Gentamicin calculated as Xn +/- 3SD (n=20) or as 90% B/Bound is equal to 0.19 ng/mL. The concentration of residue necessary to cause 50% inhibition (50% B/B_0) is approximately 1.0 ng/mL. Determinations closer to the middle of the calibration range of the test yield the most accurate results



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

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

Cross-reactivities:	Gentamicin	100%

Samples: To eliminate matrix effects in milk and honey samples, sample dilution is required. See Preparation of Samples section. For additional extraction procedures for various matrices please contact Abraxis LLC.

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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Gentamicin ELISA, Microtiter Plate

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

Product No. 5111GEN1A

1. General Description

The Gentamicin ELISA is an immunoassay for the detection of Gentamicin. This test is suitable for the quantitative and/or qualitative detection of Gentamicin in contaminated samples. Positive samples 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 Gentamicin in a basic solution. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of standard and stopping solutions with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The Gentamicin ELISA Kit should to 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 test is a direct competitive ELISA based on the recognition of Gentamicin by specific antibodies. Gentamicin, when present in a sample, and a Gentamicin-enzyme conjugate compete for the binding sites of anti-Gentamicin antibodies which are immobilized on the wells of the microtiter plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of Gentamicin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Gentamicin 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 Gentamicin ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.), positive samples requiring some action should be confirmed by an alternative method.

Working Instructions

A. Materials Provided

1. Microtiter plate coated anti-Gentamicin antibody, in a resealable foil pouch with desiccant.
2. Gentamicin Standards (6): 0, 0.25, 0.50, 1.0, 2.5 and 5.0 ng/mL.
3. Assay Buffer, 6 mL.
4. Sample Diluent (10X) Concentrate, 25 mL, must be diluted before use. Use to dilute samples.
5. Gentamicin-HRP Conjugate Solution, 12 mL.
6. Wash Solution (5X) Concentrate, 100 mL.
7. Color (Substrate) Solution (TMB), 12 mL.
8. Stop Solution, 12 mL.

B. 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 (wave length 450 nm)
4. Timer
5. Tape or Parafilm
6. Glass vials with Teflon-lined caps
7. Distilled or deionized water
8. Vortex mixer

C. 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 assay buffer, conjugate, substrate and stop solutions in order to equalize the incubations 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 should be stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standards, assay buffer, conjugate, substrate, and stop solutions are ready to use and do not require any further dilutions.
4. The standard solutions should be handled with care as they are strongly basic.
5. Dilute the sample diluent (10X) concentrate at a ratio of 1:10. If using the entire bottle (25 mL), add to 225 mL of deionized or distilled water.
6. Dilute the wash buffer (5X) concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water.
7. The stop solution should be handled with care as it contains diluted H_2SO_4.

D. Preparation of Samples

Samples should be analyzed immediately after preparation to prevent adsorption/degradation of the analyte.

Milk

1. Add 900 µL of 1X diluted Sample Diluent to an appropriately labeled glass vial with a Teflon-lined cap.
2. Add 100 µL of milk sample. Vortex thoroughly.
3. Analyze as sample (Assay Procedure, step 1).

The Gentamicin concentration contained in milk samples is then determined by multiplying the ELISA result by the dilution factor of 10. Highly contaminated samples (those outside of the calibration range of the assay) must be diluted further in 1X Sample Diluent and re-analyzed.

Honey

1. Add 1 g of honey to a clean glass vial with a Teflon-lined cap.
2. Add 4 mL of distilled or deionized water. Vortex until honey is completely dissolved.
3. Analyze as sample (Assay Procedure, step 1).

The Gentamicin concentration contained in honey samples is then determined by multiplying the ELISA result by the dilution factor of 5. Highly contaminated samples (those outside of the calibration range of the assay) must be diluted further in 1X Sample Diluent and re-analyzed.

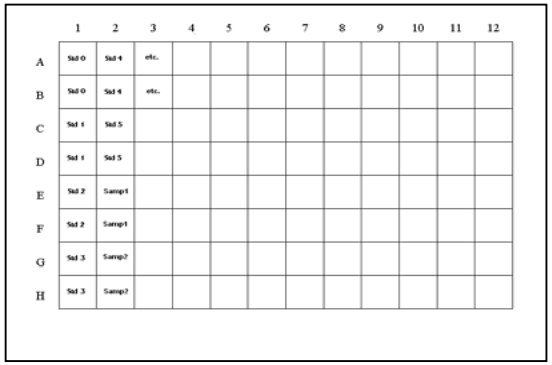
For additional extraction procedures for various matrices please contact Abraxis LLC.

E. 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.

Std 0-Std 5: Standards
0; 0.25; 0.50; 1.0; 2.5; 5.0 ppb

Samp1, Samp2, etc.: Samples



F. Assay Procedure

1. Add 25 µL of **assay buffer solution** to the individual wells successively using a multi-channel pipette or a stepping pipette.
2. Add 25 µL of the **standard solutions and samples or sample extracts** into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
3. Add 100 µL of **enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette.
4. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill contents.
5. Incubate the strips for 30 minutes at room temperature.
6. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Wash the strips **three times** using the 1X washing buffer solution. Use 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
7. Add 100 µL of **substrate (color) solution** to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Incubate the strips for 15 minutes at room temperature. Protect the strips from direct sunlight.
8. Add 100 µL of **stop solution** to the wells in the same sequence as for the substrate solution.
9. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

G. 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 $\%B/B_0$ 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 $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding Gentamicin concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb of Gentamicin by interpolation using the standard curve. Samples showing lower concentrations of Gentamicin compared to Standard 1 (0.25 ng/mL) should be reported as containing < 0.25 ng/mL. Samples showing a higher concentration than Standard 5 (5.0 ng/mL) must be diluted further to obtain accurate results.