Gentamicin ELISA Kit

Enzyme Immunoassay for the quantification of Gentamicin in serum, plasma (heparin, citrate), cell lysate, tissue homogenates and food samples.

Catalog number: ARG82660

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Gentamicin, sold under brand name Garamycin among others, is an antibiotic used to treat several types of bacterial infections. This may include bone infections, endocarditis, pelvic inflammatory disease, meningitis, pneumonia, urinary tract infections, and sepsis among others. It is not effective for gonorrhea or chlamydia infections. It can be given intravenously, by injection into a muscle, or topically. Topical formulations may be used in burns or for infections of the outside of the eye. In the developed world, it is often only used for two days until bacterial cultures determine what specific antibiotics the infection is sensitive to. The dose required should be monitored by blood testing.

Gentamicin can cause inner ear problems and kidney problems. The inner ear problems can include problems with balance and hearing loss. These problems may be permanent. If used during pregnancy, it can cause harm to the developing baby. However, it appears to be safe for use during breastfeeding. Gentamicin is a type of aminoglycoside. It works by disrupting the ability of the bacteria to make proteins, which typically kills the bacteria.

Gentamicin was patented in 1962 and approved for medical use in 1964. It is made from the bacterium *Micromonospora purpurea*. It is on the World Health Organization's List of Essential Medicines. The World Health Organization classifies gentamicin as critically important for human medicine. It is available as a generic medication. [Provide by Wikipedia: Gentamicin]

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. A highly purified Gentamicin Conjugate has been pre-coated onto a Protein Binding plate. The samples or Standards (Gentamicin Standards) are first added to the Gentamicin Conjugate pre-coated microplate. After a brief incubation, an Antibody Conjugate specific for Gentamicin is added to each well and incubate. The Gentamicin Conjugate competes with the samples / Standards for the limited number of antibody sites. After washing away any unbound antibody, a HRP Conjugate Antibody added to the wells. After washing away any unbound substances, the TMB Substrate is added to the wells and color develops in inversely proportion to the amount of Gentamicin content bound with the Antibody Conjugate. The color development is stopped by the addition of STOP Solution and the intensity of the color is measured at a wavelength of 450nm. The concentration of Gentamicin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, aliquot and store Antibody Conjugate (500X), HRP Conjugate (1000X), Standards, 500X Gentamicin Conjugate, and 100X Conjugate Diluent at -20°C to avoid multiple freeze/thaw cycles. Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Protein Binding microplate	8 X 12 strips	4°C
Standards (150 µM Gentamicin)	50 µL	-20°C
500X Gentamicin Conjugate	25 µL	-20°C
100X Conjugate Diluent	300 µL	-20°C
Diluent Buffer	50 ml (ready to use)	4°C
Antibody Conjugate (Anti-Gentamicin Antibody, 500X)	10 µL	-20°C
HRP Conjugate (1000X)	20 µL	-20°C
10X Wash Buffer	100 ml	4°C
TMB Substrate	12 ml (ready to use)	4°C (protect from light)
STOP Solution	12 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Bovine Serum Albumin (BSA)
- 1X PBS
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- The Antibody Conjugate (500X), HRP Conjugate (1000X), Standards, 500X Gentamicin Conjugate, and 100X Conjugate Diluent should be aliquoted into smaller portions before use to ensure product integrity and store the aliquoted Stock Standard at -20°C. Avoid repeated freeze-thaw cycles.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.

- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Collect the plasma layer and store on ice.

Cell or tissue: Homogenize 50-200 mg of the cell pellet or tissue in 0.5-2 mL of ice cold PBS using a mortar and pestle or by dounce homogenization. Incubate the homogenate at 4°C for 20 minutes. Transfer the homogenate to a centrifuge tube and centrifuge at 12000 x g for 20 minutes. Recover the supernatant and transfer to a fresh tube. Store re-suspended sample at-20°C or colder. Perform dilutions in Diluent Buffer as necessary.

Food samples: Homogenize 1-5 grams in ice cold PBS using a mortar and pestle or by dounce homogenization. Transfer the homogenate to a centrifuge tube and centrifuge at 12000 x g for 20 minutes. Store homogenized sample at-20°C or colder. Perform dilutions in Diluent Buffer as necessary.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. Aliquot samples for testing and store at -80°C. Avoid repeated freeze-thaw cycles. Perform dilutions in Diluent Buffer as necessary.
4. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- Gentamicin Conjugate coated microplate:
  1. Immediately before use, prepare 1X Conjugate Diluent by diluting the 100X Conjugate Diluent in 1X PBS. Example: Add 50 µL to 4.95 mL of 1X PBS.
  2. Immediately before use, prepare 1X Gentamicin Conjugate by diluting the 500X Gentamicin Conjugate in 1X Conjugate Diluent. Example: Add 10 µL of 500X Gentamicin Conjugate to 4.99 mL of 1X Conjugate Diluent.
  3. Add 100 µL of the 1X Gentamicin Conjugate to each well to be tested and incubate overnight at 4°C. Remove the Gentamicin Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Diluent Buffer to each well and block for 1 hour at room temperature on a microplate shaker. Transfer the plate to 4°C and remove the Diluent Buffer immediately before use.

  Note: The Gentamicin Conjugate coated wells are not stable and should be used within 24 hours after coating. Only coat the number of wells to be used immediately.
- 1X Wash Buffer: Dilute 10X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 50 ml of 10X Wash Buffer into 450 ml of distilled water to a final volume of 500 ml) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C.
- Antibody Conjugate and HRP Conjugate: Immediately before use dilute the Antibody Conjugate (Anti-Gentamicin Antibody) 1:500 and the HRP

Conjugate 1:1000 with Diluent Buffer. Do not store diluted solutions.

- **Standards (Gentamicin standards):** Prepare a dilution series of Gentamicin Standards in the concentration range of 0 to 1500 nM by diluting the Gentamicin Standards to the suggested concentration table below:

Standard tubes	Final Gentamicin (nM)	Diluent Buffer (µL)	Standards (µL)
S1	1500	495	5 of 150 µM Standards
S2	750	200	200 of S1
S3	375	200	200 of S2
S4	188	200	200 of S3
S5	94	200	200 of S4
S6	47	200	200 of S5
S7	23	200	200 of S6
S0	0	200	0

Note: Dilutions for the standard must be made and applied to the plate immediately. S0 serves as background.

ASSAY PROCEDURE

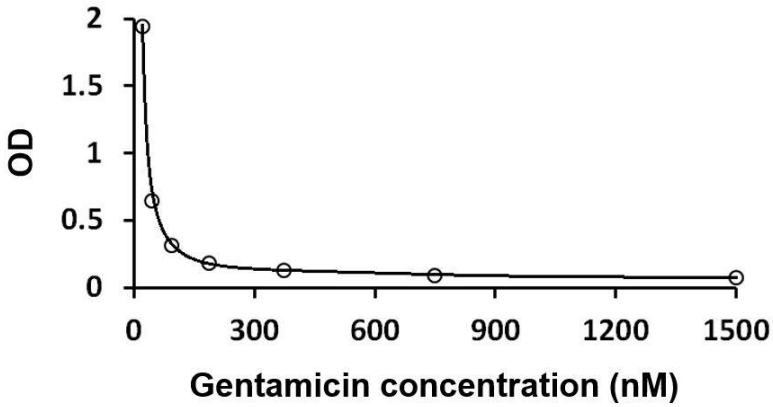
All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Each Gentamicin samples or Standards should be assayed in duplicate or triplicate.

1. Add **50 µL** of **sample or Standards** to the Gentamicin Conjugate coated microplate.
2. Incubate at **RT** for **10 minutes** on a microplate shaker.
3. Add **50 µL** of the **diluted Antibody Conjugate** to each well, incubate at **RT** for **1 hour** on a microplate shaker.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with **1× PBS (250 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µL** of the **diluted HRP Conjugate** to each well. Incubate at **RT** for **1 hour** on a microplate shaker.
6. Aspirate each well and **wash as step 4**.
7. Warm **TMB Substrate** to **RT**. Add **100 µL** of **TMB Substrate** to each well, including the blank wells. Incubate for **2-20 minutes** at **RT** in the dark.
   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
8. Add **100 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at **450nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the

absorbance within 30 minutes after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Gentamicin ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV value of intra-assay and inter-assay precision was ≤ 10%.

Sensitivity

23 nM