Kanamycin ELISA Kit

Kanamycin ELISA Kit is an Enzyme Immunoassay kit for the quantification of Kanamycin in urine, serum, cell or tissue lysate and food sample.

Catalog number: ARG83361

Package: 96 wells

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

Kanamycin A, often referred to simply as kanamycin, is an antibiotic used to treat severe bacterial infections and tuberculosis. It is not a first line treatment. It is used by mouth, injection into a vein, or injection into a muscle. Kanamycin is recommended for short-term use only, usually from 7 to 10 days. As with most antibiotics, it is ineffective in viral infections.

Common side effects include hearing and balance problems. Kidney problems may also occur. Kanamycin is not recommended during pregnancy as it may harm the baby. It is likely safe during breastfeeding. Kanamycin is in the aminoglycoside family of medications. It works by blocking the production of proteins that are required for bacterial survival.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. After coated Kanamycin Conjugate onto a microtiter plate antibody, Kanamycin of a sample competes with a Kanamycin-antibody for binding to the coated conjugate. After incubation the unbound antibody is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of Kanamycin in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of Kanamycin. Kanamycin concentration in the sample is calculated through a calibration curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store 100X Kanamycin Conjugate, 500X Conjugate Diluent, Standard, 500X Kanamycin Antibody at ≤ -20°C, Store other component at 2-8°C at all times. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C.
500X Kanamycin Conjugate	1 vial (25 µL)	-80°C
100X Conjugate Diluent	1 vial (300 µL)	4°C
Standard	1 vial (100 µL; 50 µM)	-20°C
500X Kanamycin Antibody	1 vial (10 µL)	4°C
1000X HRP-Streptavidin Solution	1 vial (20 µL)	4°C
Diluent Buffer	50 mL	4°C
10X Wash Buffer	100 mL	4°C
TMB Substrate	12 mL	4°C (Protect from light)
Stop Solution	12 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 450 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir
- Automated microplate washer (optional)
- 1X PBS

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store 100X Kanamycin Conjugate, 500X Conjugate Diluent, Standard, 500X Kanamycin Antibody at ≤ -20°C, Store other component at 2-8°C at all times.. Use the kit before expiration date.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature.
- 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. 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.
- 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.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Cells or tissues: 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. 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 resuspended sample at-20°C or colder. Perform dilutions in Assay Diluent as necessary.

Food samples: Homogenize 1-5 grams 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 Assay Diluent as necessary.

REAGENT PREPARATION

- **1X Conjugate Diluent:** 10 minutes before use, dilute **100X** Kanamycin Conjugate into 1X PBS to yield 1X Kanamycin Conjugate.
- **1X Kanamycin Conjugate**: 10 minutes before use, dilute **500X** Kanamycin Conjugate into 1X Conjugate Diluent to yield 1X Kanamycin Conjugate.
- **Kanamycin Conjugate Coated Plate**: Add 100 µL Kanamycin conjugate coating solution to each well and incubate overnight at 4°C.

  After incubation, remove the Kanamycin conjugate coating solution and wash once with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature. Transfer the plate to 4°C and remove the Assay Diluent immediately before use.
- **1X Wash Buffer:** Dilute **10X** Wash Buffer into distilled water to yield 1X Wash Buffer.
- **1X Kanamycin Antibody:** 10 minutes before use, dilute **500X** Kanamycin Antibody into Diluent Buffer to yield **1X** Kanamycin Antibody.
- **1X HRP-Streptavidin Solution:** 10 minutes before use, dilute **1000X** HRP-Streptavidin Solution into Diluent Buffer to yield **1X** HRP-Streptavidin Solution. Keep diluted HRP-Streptavidin Solution in dark before use.
- **Standard:** Centrifuge the un-reconstituted standard at 6000 x g for 1 minute to bring down the material prior to open the vial. The Diluent Buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Diluent Buffer. Diluted the standard as below:

Standard tube	Kanamycin (µM)	Diluent Buffer (µL)	Standard (µL)
S1	500	990	10 (50 µM Stock)
S2	250	500	500 of S1
S3	125	500	500 of S2
S4	62.5	500	500 of S3
S5	31.3	500	500 of S4
S6	15.6	500	500 of S5
S7	7.8	500	500 of S6
S0	0	500	0

Note: Working standard should be prepared immediately prior to use.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

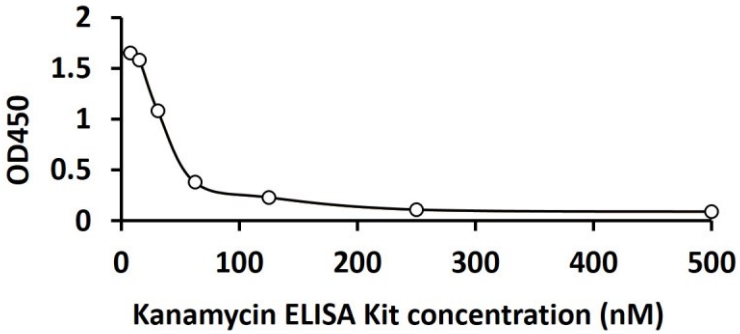
1. Add **50 µL** of **Samples, Standard** into respective wells of the 96-well plate.
2. Cover the plate and incubate for **10 min** at **RT**.
3. Add **50 µL** of **1X Kanamycin antibody** into each wells.
4. Cover the plate and incubate for **1 hour** at **RT**.
5. Aspirate each well and wash, repeating the process 2 time for a **total 3 washes.** Wash by filling each well with **1X Wash Buffer (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.
6. Add **100 µL** of **1X HRP-Streptavidin Solution** to each well.
7. Cover the plate and incubate for **1 hour** at **room temperature** in the dark.
8. Aspirate each well and **wash** plate **as step 5**.
9. Add **100 µL** of **TMB Substrate** in each well.
10. Incubate for **5-30 mins** at **room temperature** in the dark.
11. Add **100 µL** of **Stop Solution** to each well to stop the reaction.
12. Read the absorbance with a plate reader at **O.D. 450 nm.** It is recommended reading the absorbance within 10 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control 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. Using 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 details. (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.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Kanamycin ELISA Kit. One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

5 nM

Assay Range

7.8 - 500 nM