Product Manual

Gentamicin Competitive ELISA Kit

Catalog Number

MET-5135 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Gentamicin is an antibiotic that has been used to treat various types of bacterial infections such as pelvic inflammatory disease, pneumonia, bone infections, endocarditis, meningitis, sepsis, and urinary tract infections. Gentamicin was patented in 1962 and approved to be used medically in 1964. Gentamicin can be administered topically, intravenously, or by injection into a muscle. Gentamicin is an aminoglycoside that works by blocking synthesis of bacterial proteins resulting in bacterial death. This antibiotic is made from the bacterium Micromonospora purpurea.

As a side effect of treatment, gentamicin can cause inner ear problems (such as hearing loss or poor balance) as well as kidney problems. In addition, gentamicin can cause harm to a developing baby in pregnant women. However, the antibiotic appears to be safe when administered to women who are breastfeeding newborns. Gentamicin is also used by researchers as an antibacterial agent in cell cultures. Gentamicin is a very heat-stable antibiotic, remaining active even after autoclaving, which makes the antibiotic useful in the manufacturing of some types of microbiological growth media.

Cell Biolabs' Gentamicin Competitive ELISA Kit provides a convenient method for the detection of total Gentamicin in extracts from cells, tissue, serum, plasma, urine, or foods. The total content of gentamicin in unknown samples is determined by comparison with a gentamicin standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples. The kit has a detection sensitivity limit of 25 nM gentamicin.

Assay Principle

First, a gentamicin conjugate is coated on an ELISA plate. The unknown gentamicin samples or gentamicin standards are then added to the gentamicin conjugate preabsorbed ELISA plate. After a brief incubation, an anti-gentamicin monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The total content of gentamicin in unknown extracted samples is determined by comparison with a gentamicin standard curve.

Related Products

1. MET-5144: Kanamycin Elisa Kit (Colorimetric)
2. MET-5133: Formate Assay Kit (Colorimetric)

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-Gentamicin Antibody (500X) (Part No. 51351C): One 10 µL vial of anti-Gentamicin Antibody.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Gentamicin Standard (Part No. 51352C): One 50 µL vial of 150 µM Gentamicin.

2. Gentamicin Conjugate (500X) (Part No. 51353C): One 25 µL vial.
3. 100X Conjugate Diluent (Part No. 281603): One 300 µL vial.

Materials Not Supplied

1. 1X PBS

Storage

Upon receipt, store Anti-Gentamicin Antibody (500X), Gentamicin Standard, Gentamicin Conjugate, and 100X Conjugate Diluent at -20ºC. Store all the remaining components at 4°C.

Preparation of Reagents

- Gentamicin Conjugate Coated Plate:

Note: The Gentamicin Conjugate coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.

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 4ºC. 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 Assay Diluent to each well and block for 1 hr at room temperature on an orbital shaker. Transfer the plate to 4ºC and remove the Assay Diluent **immediately before use.**

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-Gentamicin Antibody and Secondary Antibody: Immediately before use, dilute the Anti-Gentamicin antibody 1:500 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of Gentamicin standards in the concentration range of 0 to 1500 nM by diluting the Gentamicin Standard in Assay Diluent (Table 1).

Standard Tubes	150 µM Gentamicin Standard (µL)	Assay Diluent (µL)	Gentamicin (nM)
1	5	495	1500
2	200 of Tube #1	200	750
3	200 of Tube #2	200	375
4	200 of Tube #3	200	188
5	200 of Tube #4	200	94
6	200 of Tube #5	200	47
7	200 of Tube #6	200	23
8	0	200	0

Table 1. Preparation of Gentamicin Standards

Preparation of Samples

- Serum: Avoid hemolyzed and lipemic blood samples. 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. Remove the yellow serum supernatant without disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in Assay Diluent as necessary.
- Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood with heparin or citrate and centrifuge at 2000 x g and 4ºC for 10 minutes. Remove the plasma layer and store on ice. Avoid disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in Assay Diluent as necessary.
- 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. 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 resuspended sample at -20°C or colder. Perform dilutions in Assay Diluent 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 Assay Diluent as necessary.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each Gentamicin sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or Gentamicin standard to the wells of the Gentamicin Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of the diluted anti-Gentamicin antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash 3 times with 250 µL of 1X Wash Buffer with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 4 above.
6. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well. Incubate at room temperature for 2-30 minutes on an orbital shaker.

Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

7. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
8. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Example of Results

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

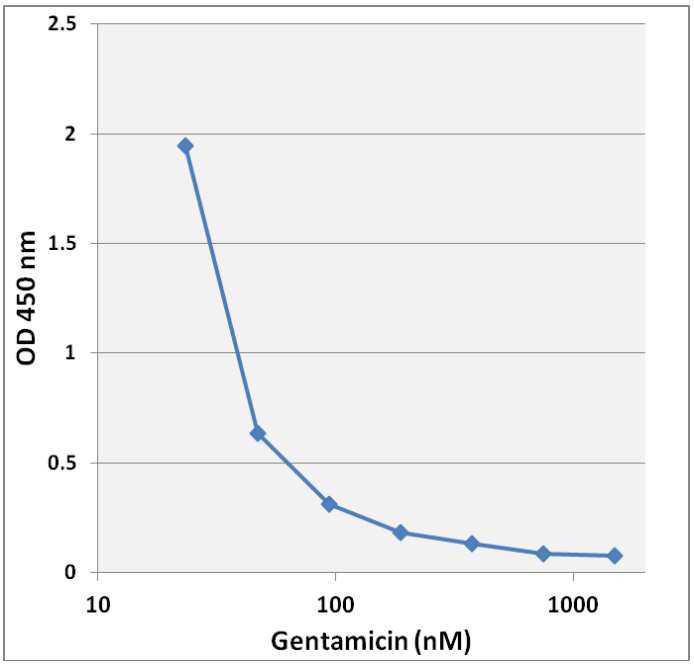

Figure 1: Gentamicin Standard Curve.

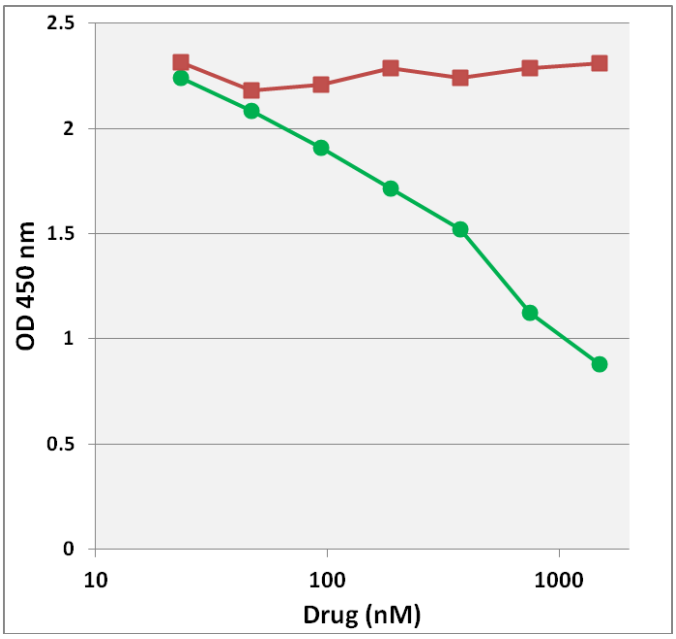

Figure 2: Specificity of Gentamicin ELISA. G418 (green circles) or Neomycin (red squares) was measured using the Gentamicin Competitive ELISA Kit.

References

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Recent Product Citation

Wijers, C.D.M. et al. (2022). Gram-negative bacteria act as a reservoir for aminoglycoside antibiotics that interact with host factors to enhance bacterial killing in a mouse model of pneumonia. *FEMS Microbes*. doi: 10.1093/femsmc/xtac016.

Warranty

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