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**Product Manual**

# **Kanamycin Competitive ELISA Kit**

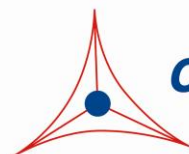
**Catalog Number**

**MET-5144**

**96 assays**

**FOR RESEARCH USE ONLY**  
**Not for use in diagnostic procedures**

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**CELL BIOLABS, INC.**  
*Creating Solutions for Life Science Research*

## **Introduction**

Kanamycin (also known as Kanamycin A) is an antibiotic used in the treatment of dangerous bacterial infections such as tuberculosis. It is typically administered by mouth, injection into a vein, or injection into a muscle. Kanamycin is recommended for short-term dosing from 7 to 10 days and is ineffective in viral infections. Common side effects include problems with hearing and balance as well as kidney problems. Kanamycin belongs to the aminoglycoside family of medications and it blocks the production of proteins that are required for bacterial survival. Specifically, it binds to the bacterial ribosomal 30S subunit which results in improper alignment with mRNA and causes the wrong amino acid to be synthesized into the peptide. As a result, nonfunctional peptide chains are produced leading to bacterial death.

Kanamycin is commonly used as a selective agent by molecular biologists to grow bacteria (e.g., *E. coli*) which have taken up expression plasmids (circular DNA) also containing a gene that codes for kanamycin resistance (usually Neomycin phosphotransferase II [NPT II/Neo]). Bacteria that have taken up and express the exogenous kanamycin resistance gene are grown on kanamycin containing luria broth (LB) agar plates or are grown in liquid LB media containing kanamycin. Only the bacteria expressing the kanamycin resistance gene can resist the selection conditions and grow.

Cell Biolabs' Kanamycin Competitive ELISA Kit provides a convenient method for the detection of total kanamycin in extracts from cells, tissue, serum, plasma, or foods. The total content of kanamycin in unknown samples is determined by comparison with a kanamycin 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 7.8 nM kanamycin.

## **Assay Principle**

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

## **Related Products**

1. MET-5135: Gentamicin Competitive ELISA Kit
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-Kanamycin Antibody (500X) (Part No. 51441C): One 10  $\mu$ L vial of anti-Kanamycin Antibody.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20  $\mu$ L 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. Kanamycin Standard (Part No. 51442C): One 100  $\mu$ L vial of 50  $\mu$ M Kanamycin.
2. Kanamycin Conjugate (500X) (Part No. 51443C): One 25  $\mu$ L vial.
3. 100X Conjugate Diluent (Part No. 281603): One 300  $\mu$ L vial.

### **Materials Not Supplied**

1. 1X PBS

### **Storage**

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

### **Preparation of Reagents**

- Kanamycin Conjugate Coated Plate:

*Note: The Kanamycin 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.*

1. Immediately before use, prepare 1X Conjugate Diluent by diluting the 100X Conjugate Diluent in 1X PBS. Example: Add 50  $\mu$ L to 4.95 mL of 1X PBS.
  2. Immediately before use, prepare 1X Kanamycin Conjugate by diluting the 500X Kanamycin Conjugate in 1X Conjugate Diluent. Example: Add 10  $\mu$ L of 500X Kanamycin Conjugate to 4.99 mL of 1X Conjugate Diluent.
  3. Add 100  $\mu$ L of the 1X Kanamycin Conjugate to each well to be tested and incubate overnight at 4°C. Remove the Kanamycin Conjugate coating solution and wash twice with 200  $\mu$ L of 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200  $\mu$ L of Assay Diluent to each well and block for 1 hr at room temperature on an orbital shaker. Transfer the plate to 4°C 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-Kanamycin Antibody and Secondary Antibody: Immediately before use, dilute the Anti-Kanamycin antibody 1:500 or Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

### **Preparation of Standard Curve**

Prepare a dilution series of Kanamycin standards in the concentration range of 0 to 500 nM by diluting the Kanamycin Standard in Assay Diluent (Table 1).

Standard Tubes	50 $\mu$ M Kanamycin Standard ( $\mu$ L)	Assay Diluent ( $\mu$ L)	Kanamycin (nM)
1	10	990	500
2	500 of Tube #1	500	250
3	500 of Tube #2	500	125
4	500 of Tube #3	500	62.5
5	500 of Tube #4	500	31.3
6	500 of Tube #5	500	15.6
7	500 of Tube #6	500	7.8
8	0	500	0

**Table 1. Preparation of Kanamycin 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 -80°C. 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 4°C for 10 minutes. Remove the plasma layer and store on ice. Avoid disturbing the white buffy layer. Aliquot samples for testing and store at -80°C. 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. 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.

### **Assay Protocol**

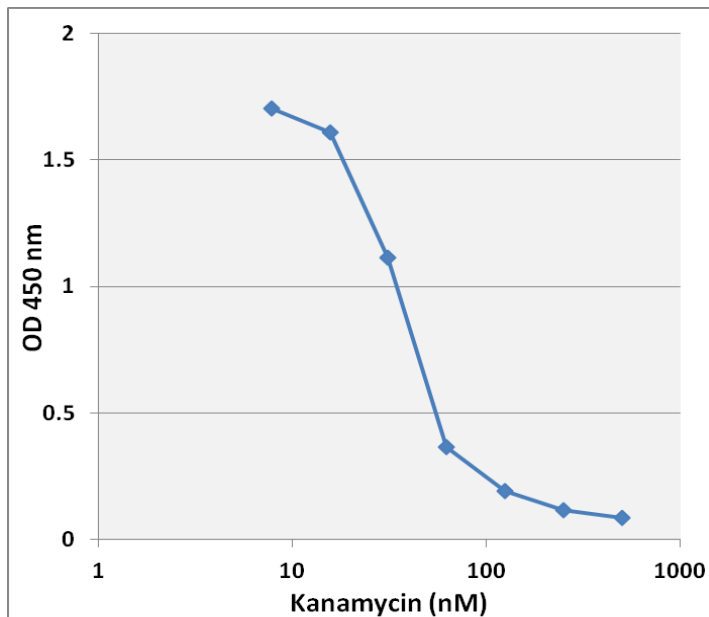
1. Prepare and mix all reagents thoroughly before use. Each Kanamycin sample including unknown and standard should be assayed in duplicate.
2. Add 50  $\mu$ L of unknown sample or Kanamycin standard to the wells of the Kanamycin Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50  $\mu$ L of the diluted anti-Kanamycin antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash 3 times with 250  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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.*

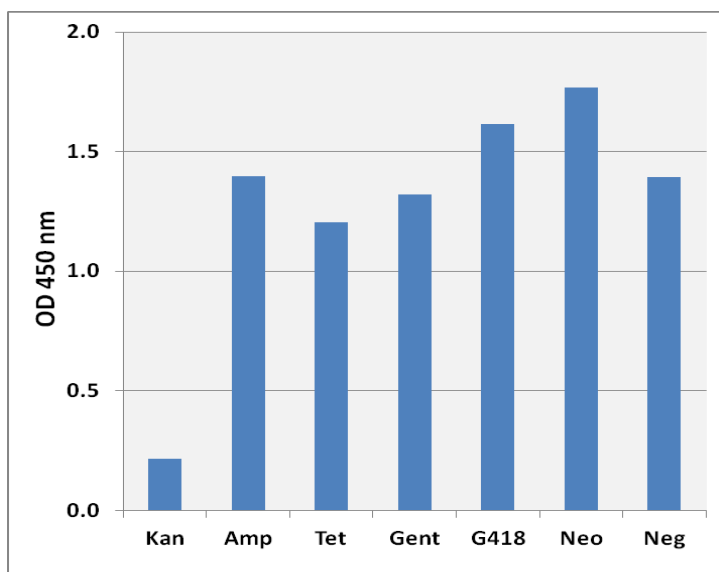
7. Stop the enzyme reaction by adding 100  $\mu\text{L}$  of Stop Solution to each well. Results should be read immediately (color will fade over time).
8. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

### **Example of Results**

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



**Figure 1: Kanamycin Standard Curve.**



**Figure 2: Specificity of Gentamicin ELISA.** Kanamycin (Kan), Ampicillin (Amp), Tetracycline (Tet), Gentamicin (Gent), Geneticin (G418), and Neomycin (Neo) were tested at 62.5 nM or 0 nM (Neg) using the Kanamycin Competitive ELISA Kit.

## **References**

1. Hotta K and Kondo S (2018). *J. Antibiot. (Tokyo)*. **71**: 417-424.
2. Bunn PA (1970) *Med Clin North Am.* **54**:1245-1256.
3. Pindell MH (1966) *Ann N Y Acad Sci* **132**: 805-810.
4. Hieber JP and Nelson JD. (1976) *Antimicrob. Agents Chemother.* **9**:899-902.

## **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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