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Gentamicin (serum/urine) ELISA Kit

rev.09/19

(Catalog # K4315-100; 100 assays, Store kit at -20°C)

I. Introduction:

Gentamicin, an aminoglycosidic antibiotic, has a broad spectrum towards most bacteria, particularly gram-negative species. It is widely used by veterinaries, has industrials applications as an additive and can be found on foodstuff consumed by animals. Its popularity is due to its broad antibacterial spectrum, efficacy and low cost. It is also known that unregulated and excessive use of this antibiotic causes negative effects in humans (i.e. neurological, hearing and renal damage). Due to its dangerous side effects, the FDA has established strict parameters that are considered safe for human consumption. Consequently, monitoring Gentamicin concentration amounts in animal-derived food is critical. Standard techniques/instruments (HPLC or GC-MS) are utilized to detect Gentamicin. However, these techniques are complex, expensive, laborious, and time-consuming. Immunoassay techniques, such as ELISAs are commonly preferred as a simple, reliable and rapid method for the quantification of Gentamicin in various samples. BioVision's Gentamicin ELISA Kit is a competitive-based ELISA that can be used for the determination of this antibiotic in dairy products, mammalian tissues and human biofluids. This Gentamicin detection kit is fast, can be completed within 90 min, offers ready-to-use reagents and can detect Gentamicin within a range between 0.3 to 24.3 ppb (ng/ml).

II. Applications:

In vitro, quantitative determination of gentamicin
Detection Range: 0.3 - 24.3 ppb (ng/ml)
Sensitivity: 0.3 ppb
Detection limit: 3 ppb for Milk and Tissue

III. Sample Type:

Serum, urine, milk and tissues (i.e. pork, liver, chicken, fish and shrimp)

IV. Kit Contents:

Components	K4315-100	Cap Code	Part Number
ELISA Microplate	8 X 12 Strips	--	K4315-100-1
Gentamicin Standards (S0 – S5)	1 ml X 6	Various	K4315-100-2.x
HRP Conjugate Stock	10 µl	Blue	K4315-100-3
Antibody	7 ml	NM/Red	K4315-100-4
TMB substrate	12 ml	Amber	K4315-100-5
Stop Solution	10 ml	NM/Blue	K4315-100-6
Sample Diluent	20 ml	NM	K4315-100-7
Wash Buffer (10X)	50 ml	NM	K4315-100-8
Extraction Solution	2 ml	Brown	K4315-100-9
Conjugate Buffer	7.5 ml	NM/Green	K4315-100-10
Plate Sealers	4	--	K4315-100-11

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm and 650 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards or sample dilutions

VI. Storage and Handling:

The entire kit may be stored at -20°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at -20°C.

VII. Reagent and Sample Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Antibody, TMB Substrate, Stop Solution, Sample Diluent, Extraction Solution and Conjugate Buffer:** Ready to be used. After use, store them at 4°C.
- **HRP Conjugate Stock:** Spin briefly before opening the tube. Pipet 8 µl of HRP Conjugate Stock into Conjugate Buffer (7.5 ml) bottle to prepare conjugate working solution. Vortex the bottle for a minute. The conjugate working solution is stable at 4°C for 2 months.
- **Wash Buffer (10X)**: Bring bottle to room temperature. If crystals are present, warm up to room temperature and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X solution can be stored at 4°C for one month.
- **Standards**: Ready to use.

Standards	S0	S1	S2	S3	S4	S5
Concentrations (ppb)	0	0.3	0.9	2.7	8.1	24.3

VIII. Sample Preparation:

- **Serum**
  1. Add 10 µl of Extraction Solution to 100 µl of serum and vortex well.
  2. Centrifuge the serum sample at 10,000 x g for 20 min at 4°C and recover the supernatant.
  3. Dilute the supernatant 5 fold with Sample Diluent. For example, mix 50 µl of supernatant with 200 µl of Sample Diluent.

4. Use 50 µl per well for the assay.
   Note: Dilution factor: 5

- **Urine**
  1. Centrifuge 0.5 ml of urine at 10,000 x g for 5 min and recover the supernatant.
  2. Dilute the supernatant 5 fold with Sample Diluent. For example, mix 100 µl of urine with 400 µl of Sample Diluent.
  3. Use 50 µl per well for the assay.
     Note: Dilution factor: 5
- **Milk**
  1. Add 20 µl of Extraction Solution to 1 ml of milk and vortex well.
  2. Centrifuge the sample at 10,000 x g for 20 min at 4°C and recover the clear supernatant.
  3. Dilute the supernatant 5 fold with Sample Diluent. For example, mix 100 µl of the supernatant with 400 µl of Sample Diluent.
  4. Use 50 µl per well for the assay.
     Note: Dilution factor: 5
- **Tissue (pork, liver, chicken, fish and shrimp)**
  1. Weigh 1 g of the tissue sample. Mix the tissue with 1 ml of water and 20 µl of Extraction Solution. Homogenize and vortex for 5 min.
  2. Centrifuge the sample at 10,000 x g for 20 min at 4°C and recover the supernatant.
  3. Dilute the supernatant 5 fold with Sample Diluent. For example, mix 100 µl of the supernatant with 400 µl of Sample Diluent
  4. Use 50 µl per well for the assay.
     Note: Dilution factor: 5

IX. Gentamicin ELISA Assay Protocol:

Notes: It is recommended that all standards and samples should be run at least in duplicate.
Standard curves must be run each time an assay is performed.

1. Prepare all reagents, standards and samples as indicated in sections VII and VIII respectively.
2. Add 50 µl of Standards or Samples per well. Then add 50 µl of conjugate working solution and 50 µl of Antibody to the above wells.
3. Cover the microtiter plate with the plate sealer and mix well. Incubate the plate at 25°C for 30 min.
4. After incubation time, aspirate all reagents and wash each well 4 times: add 250 µl of 1X Wash Buffer and incubate for 30 seconds. Repeat this step three more times. *Complete removal of 1X Wash buffer is essential for accurate results.*
5. Add 100 µl of TMB Substrate to each well. Tap or shake the plate to ensure complete mixing.
6. Check the OD at 650 nm for the well containing no gentamicin (S0). When it reaches 0.8 – 1.0, add 50 µl of Stop Solution to each well and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm for the standards and samples immediately.

X. Calculation:

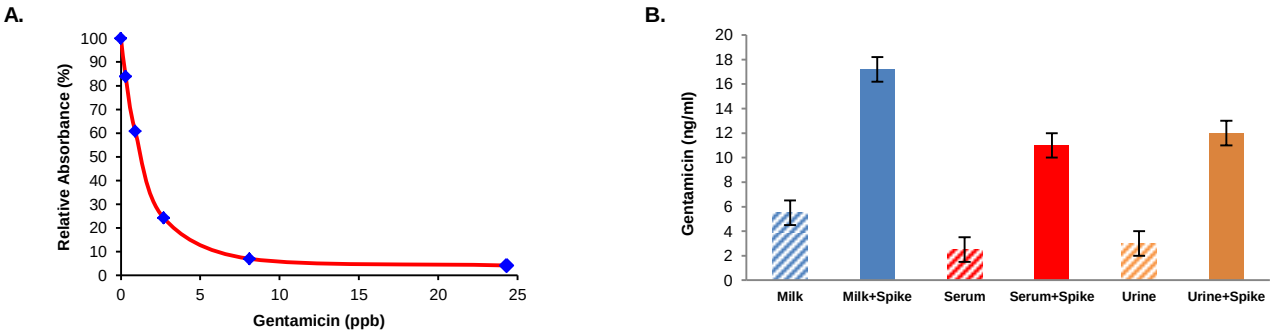
The mean values of relative absorbance for the standards or samples are divided by the absorbance value of the zero-standard (S0) and multiplied by 100%. The zero-standard is set to 100% and the relative absorbances of the standards and samples (A) are expressed as percentages.

Relative Absorbance (%) = A/A_0 X 100%

A: The average absorbance of the standard or sample

A_0 : The average absorbance of the zero standards

The Gentamicin Standard Curve is done by plotting the relative absorbance of the standards vs. gentamicin concentrations (ppb). The concentration of Gentamicin of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor.



Figures. **A.** Gentamicin standard curve (*This standard curve is for demonstration only. A standard curve must be run with each assay*). **B.** Spike recovery experiment: Milk, human serum and urine samples were assayed with and without spike (10 ng/ml). Experiments showed 80-100% recovery.

XI. RELATED PRODUCTS:

Ampicillin sodium (Cat. No. 2484-5G, 25G, 100G)
Colistin Sulfate (Cat. No. 9696-1G, 5G)
Carbenicillin disodium (Cat. No. 2485-1G, 5G, 10G)
Penicillin G sodium (Cat. No. 2503-100, 500)

FOR RESEARCH USE ONLY! Not to be used on humans.