Gentamicin ELISA Kit

rev 03/19

(Catalog # K4206-100, 100 assays, Store at 4°C)

I. Introduction:

Gentamicin (GEN) belongs to aminoglycoside antibiotics, playing the main role in gram-negative bacteria. It is widely used in veterinary clinical and animal feed additives because of its broad antibacterial spectrum, well curative effects and low cost. But it easily causes toxicant side effect during application process, especially in large and non-regulate using, causing Gentamicin residues exceeded in animal derived food, constituting a risk to human health, neurological, hearing and kidney damage. Current methods of detection Gentamicin, such as HPLC or GC-MS, are with sample preparation complex, expensive, difficult and time-consuming. While using the Gentamicin ELISA kit is both rapidly and accurately analysis Gentamicin residues in samples. This kit is a detection product developed based on competitive ELISA technology, with operation time as short as 50 min and a sensitivity of 0.3 ppb (ng/ml), and linear range from 0.3 ppb to 24.3 ppb (ng/ml).

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Gentamicin.
Detection Range: 0.3 - 24.3 ppb (ng/ml)
Sensitivity: 0.3 ppb (ng/ml)
Detection limitation: 3 ppb for Milk and Tissue.

III. Sample Type:

Milk, Tissues

IV. Kit Contents:

Components	K4206-100	Part No.
Micro ELISA Plate	8 X 12 Strips	K4206-100-1
Standard (S0 – S5)	1 ml X 6	K4206-100-2
HRP-conjugate	7 ml	K4206-100-3
Antibody	7 ml	K4206-100-4
TMB substrate	12 ml	K4206-100-5
Stop Solution	10 ml	K4206-100-6
Sample Diluent	20 ml	K4206-100-7
Wash Buffer (10X)	30 ml	K4206-100-8
Plate sealers	4	K4206-100-9

V. User Supplied Reagents and Equipment:

- Chemicals: $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$, and Trichloroacetic acid (TCA)
- Microplate reader capable of measuring absorbance at 450 nm
- 25°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes. Finish preparing reagent 10 minutes before the asay.

1. **Extraction Solution 1** (for milk use only): Weigh 5.37 g of $Na_2HPO_4 \cdot 12H_2O$ and 0.78 g of $NaH_2PO_4 \cdot 2H_2O$ to 100 ml of deionized water, mix thoroughly.
2. **Extraction Solution 2 (for tissue use only):** Dilute 3 g of TCA into 100 ml deionized or distilled water, mix well.
3. **Wash Buffer (1X):** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals are completely dissolved. Dilute 10 ml of Wash Buffer (10X) into 90 ml deionized water to prepare 100 ml of Wash Buffer (1X). Can be stored at 4℃ for one month.
4. **Standards Concentration:**

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

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

5. Sample Preparation:

Note: The prepared sample maybe stored for up to one day at 2-8°C.

- **Milk and fresh milk**
  1. Bring the milk sample to room temperature.
  2. Mix 100 µl of sample with 900 µl of **Extraction Solution 1** in a new centrifugal tube.
  3. Take 50 µl of sample for further analysis. (Dilution Factor: 10)
- **Tissue (pork, liver, chicken, fish, shrimp)**
  1. Weigh 1 g of the homogenized sample and add 5 ml of Extraction Solution 2, vortex for 5 min.
  2. Centrifuge at 4000 rpm for 10 min. Transfer 200 µl of supernatant into a new centrifugal tube.
  3. Add 200 µl of Sample Diluent, shake well and take 50 µl of sample for further analysis. (Dilution Factor: 10)

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII. Store unused wells back to 2-8°C.
2. Add 50 µl of **Standard** or **Sample** per well. Then add 50 µl of **HRP-conjugate** to each well and 50 µl of Antibody to each well. Cover the microtiter plate with a new adhesive strip and mix well, then incubate for 30 min at 25°C.
3. Aspirate each well and wash, repeating the process 4 times. Wash by filling each well with 250 µl of **Wash Buffer** using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 30 seconds, complete removal of liquid at each step is essential to good performance.
4. Add 100 µl of **TMB Substrate** to each well, mix well. Incubate for 20 minutes at 25°C. Protect from light.
5. Add 50 µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
6. Read result at 450 nm within 5 minutes.

IX. CALCULATION:

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

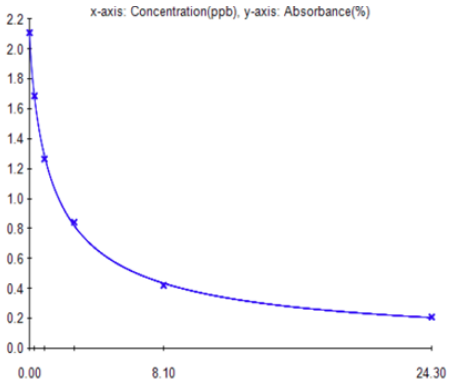
$$\text{Absorbance Value (\%)} = B/B_0 \times 100\%$$

B: The average absorbance value of the sample or standard

B_0: The average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Gentamicin standards solution (ppb) as x-axis. The Gentamicin concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.



X. RELATED PRODUCTS:

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