Kanamycin ELISA Kit

rev 03/20

(Catalog # K4210-100, 100 assays, Store at 4°C)

I. Introduction:

Kanamycin is an aminoglycoside antibiotic and is widely used in treating animal diseases. It harms the 8th cranial nerves, causing damages to the vestibular and cochlear. The main manifestations of renal toxicity are the damages of the proximal convoluted tubule, causing protein urine, hematuresis, renal hypofunction, etc. The residues of Kanamycin in animal derived food affect human health, in China and Occident, Kanamycin has been limited to use for its neurotoxicity and nephrotoxicity. This kit is a detection product developed based on competitive ELISA technology, with operation time as short as 50 min and a sensitivity of 0.5 ppb, and linear range from 0.5 ppb ~ 40.5 ppb.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Kanamycin
Detection Range: 0.5 – 40.5 ppb
Sensitivity: 0.5 ppb
Detection limitation: 5 ppb for milk and tissue, and 15 ppb milk powder

III. Sample Type:

Tissue, Milk and milk powder

IV. Kit Contents:

Components	K4210-100	Part No.
Micro ELISA Plate	8 X 12 Strips	K4210-100-1
Standard (S0 – S5)	1 ml X 6	K4210-100-2
HRP-conjugate	7 ml	K4210-100-3
Antibody	7 ml	K4210-100-4
TMB substrate	12 ml	K4210-100-5
Stop Solution	10 ml	K4210-100-6
Sample Diluent	20 ml	K4210-100-7
Wash Buffer (10X)	30 ml	K4210-100-8
Plate sealers	4	K4210-100-9

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 and graduated cylinders 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.

1. **Extraction Solution 1**: 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:** Dilute 5 ml of Sample Diluent into 95 ml deionized or distilled water, mix well.
3. **Extraction Solution 3:** Dilute 3 g of TCA into 100 ml deionized or distilled water, shake well
4. **Wash Buffer (1X):** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 10 mL of **Wash Buffer (10X)** into 90 mL deionized or distilled water to prepare 100 ml of **Wash Buffer (1X)**. Keep it at 4°C for one month.
5. **Standards Concentration:**

Standards	S0	S1	S2	S3	S4	S5
Concentration (ppb)	0	0.5	1.5	4.5	13.5	40.5

6. **Sample Preparation:**

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

A. Milk and fresh milk

1. Bring the milk sample to room temperature.

2. Transfer 100 µL of sample into a new centrifugal tube and add 900 µL of Extraction Solution 1, shake well.
3. Take 50 µL of sample for further analysis. (Dilution factor: 10)

B. Milk power

1. Weigh 1 g of milk power sample, add 5ml of Extraction Solution 2, shake properly for 5 min.
2. Add 4 ml of Extraction Solution 3, shake properly for 5 min. Centrifuge at 4000 rpm for 10 min.
3. Transfer 100 µl of supernatant into a new centrifugal tube, add 200 µl of Sample Diluent, shake well.
4. Take 50 µLof sample for further analysis. (Dilution factor: 30)

C. Tissue (chicken, pork)

1. Weigh 1 g of the homogenized tissue sample, add 10 ml of Extraction Solution 1, shake properly for 5 min. Centrifuge at 4000 rpm for 10 min.
2. Take 50 µl of supernatant 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.
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 15 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 10 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 Kanamycin standards solution (ppb) as x-axis. The Kanamycin 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.

X. RELATED PRODUCTS:

- Kanamycin Sulfate, USP (Cat. No. 2498-5G, 25G)
- Salbutamol ELISA Kit (Cat. No. K4209-100)
- Aflatoxin B1 (AFB1) ELISA Kit (Cat. No. K4208-100)
- Sulfonamides residue ELISA Kit (Cat. No. K4207-100)
- Gentamicin ELISA kit (Cat. No. K4206-100)