Kanamycin (KA) ELISA Kit

96 Wells

Catalogue Number: NEGEK0006

Valid Period: 6 months

For samples:

ELISA Kit for the quantitative Measurement of Kanamycin Residues in Samples such as Milk, Milk Powder, Tissue samples (Chicken, Pork)

FOR RESEARCH USE ONLY!

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

CONTENTS

1. BACKGROUND

Kanamycin is an aminoglycoside antibiotic widely used in the treatment of animal diseases. However, due to its neurotoxicity and nephrotoxicity, it can damage the eighth cranial nerve, leading to vestibular and cochlear impairment. Its nephrotoxic effects primarily manifest as proximal convoluted tubule damage, resulting in proteinuria, hematuria, and decreased renal function. Residual kanamycin in animal-derived food products poses a risk to human health. Therefore, regulatory authorities in the United States, European countries, and China have established strict limits on its use.

2. PRINCIPLE OF THE ASSAY

This ELISA kit employs an indirect competitive ELISA method. The microplate is pre-coated with kanamycin, and standards (or samples) along with kanamycin monoclonal antibodies are added simultaneously. The coated antigen on the microplate competes with the kanamycin in the standards (or samples) for binding to the monoclonal antibody. After incubation at room temperature, unbound components are washed away. A biotin-labeled secondary antibody is then added to bind to the kanamycin monoclonal antibody. Following another incubation and wash step, Streptavidin-HRP Conjugate is added. Finally, the reaction is developed using TMB substrate, and the absorbance of the sample is inversely correlated with the kanamycin concentration. The kanamycin concentration in the sample is determined by comparing the absorbance to the standard curve and multiplying by the corresponding dilution factor.

3. MATERIALS (Note: Store at 2-8°C)

	REAGENTS	SPECIFICATION	QUANTITY
1	Pre-Coated Microplate (Detachable)	96 wells	1 plate (Keep Sealed)
2	Kanamycin Standard (Stock Solution) -6.4μg/mL	20μL	1 vial
3.	Kanamycin Monoclonal Antibody (100×)	100μL	1 vial
4	Biotin-labeled Detection Antibody (100×)	150μL	1 vial
5	Streptavidin-HRP Conjugate (150×)	100μL	1 vial
6	Diluent Buffer (10×)	10mL	1 vial (Avoid Light)

7	TMB Substrates	10mL	1 vial
8	Wash Solution (20×)	10mL	5 vials
9	Stop Solution	10mL	1 vial
10	Plate Sealer		4 pieces
11	Instruction Manual		1

4. EQUIPMENT REQUIRED BUT NOT PROVIDED

1) 10-1000μL Pipettor and Disposable Sterilized Tips
2) Multichannel Pipettor
3) 1L Sterilized deionized Water or Ultrapure Water
4) Sterilized EP Tubes
5) Absorbent Paper
6) Microplate Reader
7) High-speed Centrifuge
8) Microplate Washer or Washing Bottles
9) Incubator or Water Bath
10) Data analysis and Graphing Software
11) Trichloroacetic acid (TCA), Tris-base

5. PREPARATION BEFORE ASSAY

1) Place all kits' components at room temperature for 30 minutes before using.
2) 1M Tris-HCl (pH 9.6): Weigh 12.114g of Tris base, dissolve it in 80mL of deionized water, adjust the pH to 9.6, and bring the final volume to 100mL with deionized water.
3) The preparation of Wash Solution (1×): Add the contents of five 10mL of Wash Solution (20×) into 950mL of deionized water or ultrapure water to prepare 1000mL of Wash Solution (1×). If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Wash Solution (1×) can be stored at 2-8°C for 2 weeks.
4) The preparation of Diluent Buffer (1×): Dilute 10mL of Diluent Buffer (10×) with 90mL of deionized water or ultrapure water to prepare 100mL of Diluent Buffer (1×). If crystals have

formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.

5) Preparation of 10mM PBS (pH:7.4): Accurately weigh 0.02g of KH_2PO_4, 0.29g of $Na_2HPO_4 \cdot 12H_2O$, 0.8g of NaCl, and 0.02g of KCl. Dissolve the salts in 80mL deionized water, then dilute to a final volume of 100mL. Mix thoroughly to ensure complete dissolution and homogeneity.

6) Preparation of 3% TCA: Weigh 3g of TCA, dissolve it in deionized water to 100mL. Mix thoroughly.

6. PREPARATION OF SPECIMEN (Note**:** Samples can be stored at 2-8°C for up to 1 day.)

1) **Milk and Fresh Milk Samples** - Heat the sample at 100°C in the metal bath or water bath for 10 minutes. After cooling to 4°C, centrifuge sample at 10,000 r/min for 10 minutes. Collect the supernatant for analysis.

2) **Milk Powder Samples** - Weigh 1.0g of skimmed milk powder, dissolve it in 5mL of PBS, and vortex to mix. Add 5mL of 3% TCA solution, vortex for 5 minutes. After cooling to 4°C, centrifuge sample at 10,000 r/min for 10 minutes. Then Take 2mL of the supernatant, add 130μL of 1M Tris-HCl buffer, and adjust the pH to 7.0-7.4. Filter through a 0.22μm membrane, and collect the filtrate for analysis.

3) **Tissue (Chicken, Pork) Samples** - Weigh 1.00±0.05g of minced tissue samples, add 10mL of PBS buffer, and vortex for 5minutes. After cooling to 4°C, centrifuge sample at 10,000r/min for 10minutes. Collect the supernatant for analysis.

4) **Other Biological Samples** - Dilute the test samples with the prepared Diluent Buffer to get a final concentration within the detection range.

7. ASSAY PROCEDURES

1) Remove the microplate from the foil bag, detach the desired number of strips from the plate, immediately reseal the remaining strips and store at 4°C. Then put the ready-for-use strips on a clean plate holder, and start the experiment after the strips returned to room temperature (**Note:**

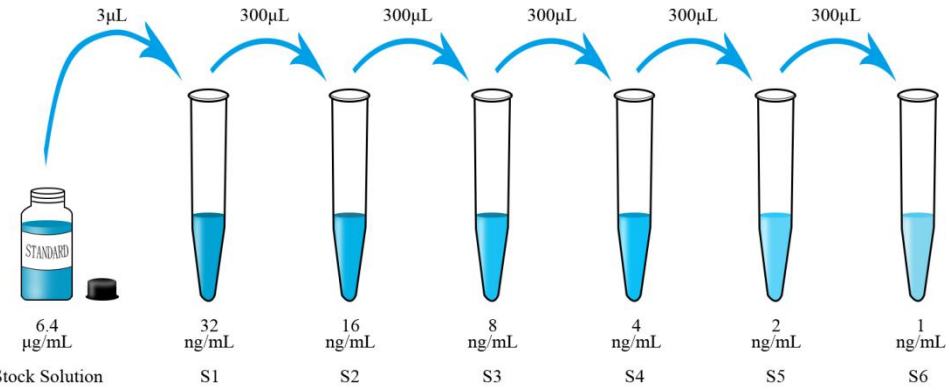
After removing the microplate, store it in a sealed and moisture-proof condition at 2-8℃ for up to 1 month. The plate holder is reusable).

2) Prepare Standards: When preparing the standards, label 6 tubes as S1, S2, S3, S4, S5, S6, and add the following volumes of Diluent Buffer (1×) to each tube: S1 (597μL), S2 to S6 (each 300μL).

Add 3μL of Standard (Stock Solution - 6.4μg/mL) into the S1. Mix gently by pipetting up and down twice, then invert to mix thoroughly.

Transfer 300μL of S1 (32ng/mL) to S2, mix gently; then transfer 300μL of the resulting S2 solution (16ng/mL) to S3, mix gently. Continue this 2-fold serial dilution sequentially through S6 to achieve a final concentration of 1ng/mL.

NOTE: There are 6 points of diluted standards, S1:32ng/mL, S2: 16ng/mL, S3: 8ng/mL, S4: 4ng/mL, S5: 2ng/mL, S6: 1ng/mL.



3) Prepare the monoclonal antibody working solution: Dilute 60μL of Kanamycin Monoclonal Antibody (100×) in 5,940μL of dilution buffer to obtain a working concentration (1×). Mix thoroughly by gently pipetting up and down and inverting the tube several times. Then, add 50μL of the diluted antibody solution to each well of the microplate.

4) Sample Addition: Add 50μL of Standard or Sample (undiluted or diluted) to each well of the microplate. For the Blank Control test, we recommend add 50μL Diluent Buffer (1×) to the well.

5) Cover the plate with a plate sealer, put it on the oscillator to mix and incubate for 90 minutes at room temperature.

6) Automated Washing:

- Put 1000mL Wash Solution (1×) into the washing bottle of the automated microplate washer for standby.
- Take out the microplate, and discard the incubation mixture. And then wash the microplate with the automated microplate washer for 5 times.
- After washing, invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.

Or Manual Washing:

- Put 1000mL Wash Solution (1×) into the washing bottle for standby.
- Take out the microplate, and discard the incubation mixture. And then invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no no moisture appears.
- Fill each well with 300μL Wash Solution (1×) by a muti-channel pipette, let stand for 20 seconds, then discard the contents, invert plate, and blot dry by hitting the plate onto absorbent paper. Repeat this washing step for 5 times.

  Note: There should be no moisture appears in the fifth washing step.

7) Addition of Biotin-labeled Detection Antibody: Take 110μL of Biotin-labeled Detection Antibody (100×) to 11mL of Diluent Buffer (1×). Gently pipette up and down and invert to mix thoroughly. After dilution to the working concentration (1×), add 100μL of the solution to each well. Seal the plate with a plate sealer and incubate at room temperature for 1 hour. Wash the plate according to Step 6.

8) Add HRP working solution (1×): Dilute 70μL of Streptavidin-HRP Conjugate (150×) with 10.5mL of Diluent Buffer (1×). Mix well by gentle pipetting and inversion to ensure complete dilution to the working concentration (1×). The add 100μL of the prepared solution to each well, seal the plate with a plate sealer, and incubate at room temperature for 30 minutes. Wash the plate according to Step 6.

9) Add 100μL of TMB substrate to each well, cover the plate with a plate sealer, and mix well by shaking for 30 seconds. Then incubate at room temperature for 10-30 minutes. (Avoid sunlight).

   Note: The coloring time varies in different experimental conditions (temperature, humidity,

ect.).

10） Add 50μL of Stop Solution to each well to stop the reaction, and gently shake the plate for 10 seconds to mix well. Immediately run the microplate reader and conduct measurement at 450nm.

8. NOTES

1) Sample Preparation

a) Samples should be frozen and aliquoted if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well by pipette or Vortex, centrifuge to remove flocculent insoluble substances before use.

b) It is recommended that all standards, controls and samples be run in duplicate. Please gently mix the liquid after adding standards and samples, and confirm there's no bubble inside.

2) Experimental Operation

a) Do not mix or interchange different ELISA kits or same ELISA kit but with different lot.

b) To avoid cross-contamination, change pipette tips between different diluted standard additions, sample additions, and reagent additions.

c) Total dispensing time for addition of reagents or samples to the plate should not exceed 3 minutes. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.

d) Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells. Do not directly touch the bottom of the microplate. If it is covered with fingerprints or stains, it will affect the measurement.

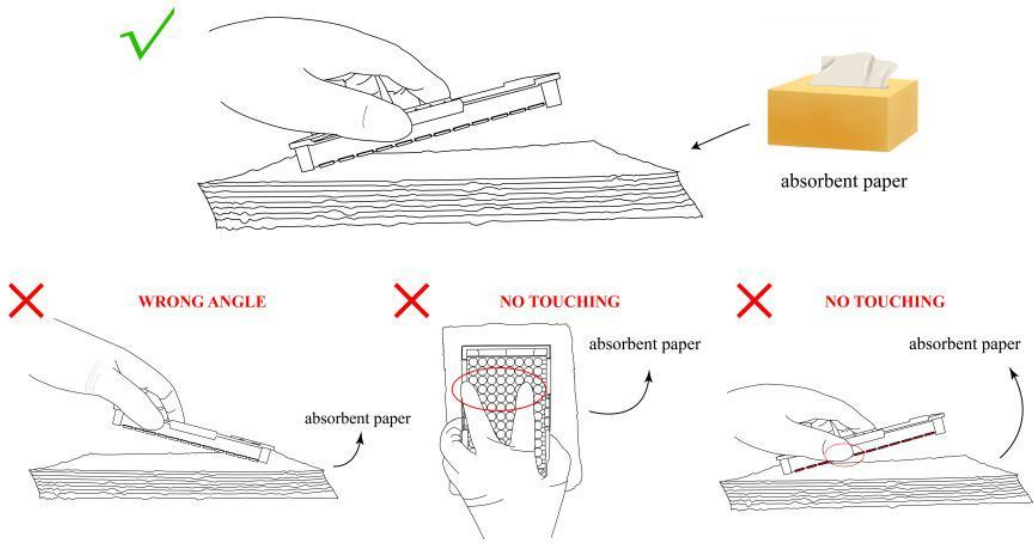
e) TMB is easily contaminated. please protect it from light and metal.

f) Monitor the reaction time. After adding TMB substrate, observe the color change of reaction wells at regular intervals (for example, about 10 minutes). If the color turns too deep, add Stop Solution in advance to terminate the reaction.

g) The Stop Solution provided with this kit is acid solution. Protective gloves, clothes, and face protection should be worn.

h) The coefficient of determination of the standard curve should be $R^2 \geqslant 0.95$.

i) Schematic diagram of tapping the plate:



9. CALCULATION OF RESULTS

1) Calculation of Percent Absorbance: The percent absorbance is calculated by dividing the mean absorbance of the standards or samples(B) by the mean absorbance of the Blank Control/S0(B0), and then multiplying by 100%. The formula is: Percent Absorbance(%)= B/B0×100%

- B: Mean absorbance of the standard or sample.
- B0: Mean absorbance of the 0-ppb standard (Blank Control).

2) Standard Curve Generation and Calculation:

Plot a four-parameter logistic (4-PL) standard curve, with the percent absorbance of the standards on the Y-axis and the logarithm of kanamycin standard concentration (ppb) on the X-axis.

3) Sample Concentration Calculation:

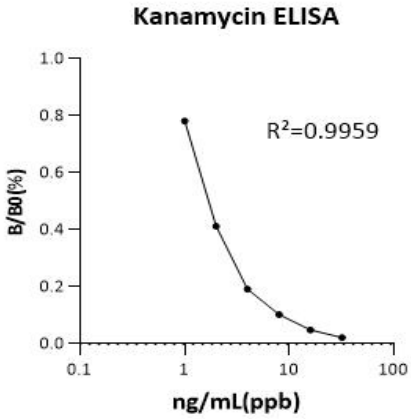
Substitute the percent absorbance of the sample into the standard curve equation to determine the corresponding concentration. Multiply the obtained concentration by the sample's dilution

factor to calculate the actual kanamycin concentration in the sample.

Note: If the OD value of S0 is less than 0.5, the kit may be deteriorated.

4) Standard curve for demonstration only.

APPENDIX 1 : EXAMPLE OF STANDARD CURVE



ng/mL	OD450nm	B/B0
32	0.063	0.022
16	0.140	0.049
8	0.295	0.103
4	0.550	0.192
2	1.179	0.413
1	2.233	0.782
0	2.856	NA

10. QUALITY CONTROL

1) Intra Variation%: 4-10.19

Inter Variation%: 7.68-9.96

2) Recovery Rate:

Sample Type	Recovery Rate (%)
Milk	80-120
Milk Powder	80-100
Tissues	80-120

3) Sensitivity: 0.5ng/mL(ppb)

4) Quantitative Detection Range: 1ng/mL(ppb)-32ng/mL(ppb)

5) Specificity/Cross-reactivity:

Sample	Cross Reactivity (%)
TOB (Tobramycin)	37.79
NEO (Neomycin)	0.02
STR (Streptomycin)	0.01
GM (Gentamicin)	0.08

7) Limitation: This ELISA kit is not suitable for the samples containing sodium azide (NaN_3). As NaN_3 is a potent inhibitor of HRP, which will reduced detection of the Aarget protein concentration.

11. SAFETY NOTES

1) This kit contains small amount of TMB (3, 3', 5, 5'- Tetramethylbenzidine) in Substrate B. TMB is non-carcinogenic but it is hazardous in case of skin contact, eye contact, ingestion and inhalation. In case of contact, rinse affected area with plenty of water.
2) The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, and face protection.

12. CONTACT US

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