UC Biodevices

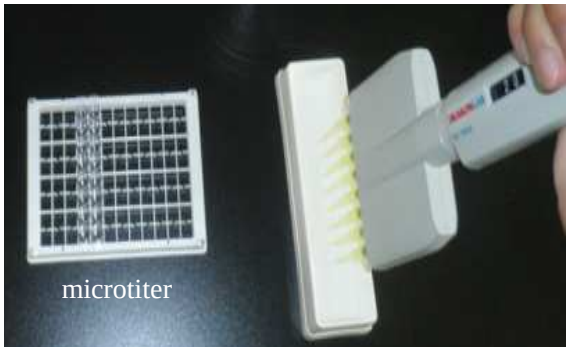
ELISA Kit for Detection of Kanamycin

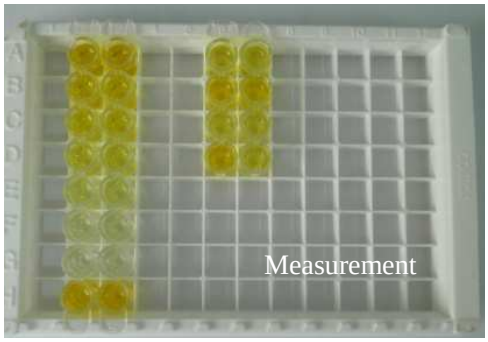
(Product Number: 5501E212)

（Kanamycin has neurological and renal toxicity; widely used in biopharmaceutical drug screening and production）

INSTRUCTION MANUAL

(v. 3.00)





1. Introduction

Kanamycin sulfate is an aminoglycoside antibiotic produced by Streptomyces kanamyceticus. Aminoglycoside antibiotic kanamycin is being widely used in animal diseases range, because of its neurotoxicity and renal toxicity, damage to the 8th cranial nerve, resulting in vestibular and cochlear damage, mainly the proximal renal toxicity Body curved pipe damage, the protein in urine, hematuria, kidney dysfunction and so on. Kanamycin can cause severe damage to the kidneys and can also cause hearing loss. Residues in animal food may affect human health, thus Europe, America and China have required their limited use. Currently, ELISA detection of kanamycin residues as a screening method has been widely used.

This manual establishes procedures for determining Kanamycin in samples including plasma/serum, cell culture supernatant, milk and processed meat products. The test is a competitive direct ELISA that provides exact concentrations in parts per billion (ppb). Free Kanamycin in the sample and standards compete with KAN Enzyme Conjugate for the Kanamycin antibody binding sites. After a wash step, substrate reacts with the bound enzyme conjugate to produce blue color.

Assay Sensitivity, Precision, Accuracy

1) Sensitivity: 1ppb
2) Precision：<20%
3) Accuracy：70-120％ average recovery rate

2. Kit Contents

1) Microtiter plate (8 wells×12) precoated with antibodies to mouse IgG.
2) Non-coated Dilution Microwells (8 wells×12 strips)
3) 5×kanamycin standard solutions (1ml/each): 0 ng/ml, 0.2 ng/ml, 0.5ng/ml, 1.0ng/ml, 3.0ng/ml. (Note: 1mg of kanamycin=775U of kanamycin, so that 1U/ml=1.29μg/ml=1290ppb)
4) KAN Enzyme Conjugate(1×) (15ml)
5) Washing buffer(10×) (50ml)
6) KAN dilution buffer (2x50ml)
7) Substrate TMB solution (17ml)

8) Stop solution (7ml)

9) Instruction Manual

Materials and reagents required but not provided:

1) 50, 100 and 200µl precision micropipette.
2) 50-200µl multichannel micropipette.
3) microtitre plate reader with 450nm filter.
4) Microwell plate mixer.
5) Timer.
6) Filter.
7) Centrifuge

3. Preparation of Working Solutions

- **Kanamycin standard solutions**: ready to use

- **KAN Enzyme Conjugate**: ready to use

- **Washing buffer**: dilute 10× with DI water (1+9) (e. g. 20.0mL 10×Wash buffer +180.0 mL DI water, sufficient for 4 microtiter strips 48 wells).

- **TMB**: ready to use.

- **Stop solution**: ready to use.

4. Preparation of Samples

4.1 Blood plasma/serum, Cell culture supernatant, Urine

a. Keep samples at 4^{o}C for overnight.
b. Transfer 1mL into a centrifugal vial
c. Centrifuge: 4000r/min, 10min.
d. Take 50µL of the supernatant above and dilute it with 450µL of KAN dilution buffer.
e. Use 50µl in the test.

The dilution factor is 20 for blood, urine and cell culture supernatant samples.

4.2 Milk

a. Transfer 5ml milk into a centrifugal vial.
b. Centrifuge: 10 min / 4000 g / room temperature (20 - 25℃ / 68 - 77℉)
c. Carefully remove 20µl of the skim portion (bottom layer) of the milk for analysis without disturbing the top fat layer and transfer sample into a 1.5ml test vial.
d. Dilute 20µl of the filtrate with 980µl of KAN dilution buffer, and mix them thoroughly.
e. Use 50µl in the test

The dilution factor is 50 for milk samples.

4.3 Meat

a. Transfer 5 g of the homogenized sample into a centrifugal screw cap vial, add 20ml pH7.4 PBS and vigorously shake for 30 min.
b. Centrifuge: 10 min/3000g / room temperature.
c. Transfer 100µl of the supernatant into a 1.5ml test vial
d. Dilute 100µl of the supernatant with 900µl of KAN dilution buffer, and mix them thoroughly.
e. Use 50µl in the test.

The dilution factor is 40 for meat samples.

4.4 Medicine

a. Take suitable amount of solid medicine (e.g., 10 mg), dissolve it in an appropriate solvent (e.g., 1 ml water or 0.01 M HCl or 0.01 M NaOH) to dissolve all solid then filter the solution (filtrate).
b. Dilute 100µl of the above filtrate or liquid medicine with 900µl of KAN diluent buffer and mix them thoroughly.
c. Use 50µl in the test.

The dilution factor is 10 for liquid medicine.

5. Immunoassay Procedure

a) Allow reagents, microwells, and sample extracts to reach room temperature prior to starting a test.

b) Insert an appropriate number of **Non-coated dilution microwells** into a microwell strip holder for all standards and samples to be tested. (One or two, if a parallel test is needed, Dilution Microwells are required for each standard or sample).

c) Insert the same number of **Antibody-coated microwells** into a strip microwell holder for all standards and samples to be tested. Place the unused microwell strips back to the foil pouch with the desiccant packet, and put the pouch into a Ziploc bag to seal it.

d) Using a new pipette tip for each standard and sample, pipette 75µl of standards and prepared sample to separate **Non-coated dilution microwells**.

e) Add 75µl of enzyme conjugate into each **Non-coated dilution microwell**.

f) Mix each well by carefully pipetting it up and down 5 times and immediately transfer 100 µL of the solution from each Dilution Microwell into a corresponding **Antibody Coated Microwell**

g) Incubate for 30 minutes at room temperature

h) Dump the solutions of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.

i) Fill each well with the washing buffer (ca. 250 ul). Empty the wells again and remove all remaining liquid. Repeat this step 4 times. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.

j) Add 100µl of TMB to each well.

k) Incubate for 10 more minutes at room temperature. Cover the wells with a paper towel to protect them from light sources.

l) Add 50µl of stop solution to each well, mix thoroughly.

m) Read results using a microwell reader with a 450 nm filter at a wavelength of 630 nm.

6. Calculation

Divide the mean absorbance value of standards or samples (B_i) by the mean absorbance value of the Maximum Binding (B_0) and multiply by 100. Maximum binding is thus made equal to 100% and the absorbance values are quoted in percentages:

absorbance standard (or sample)

---------- × 100 = B_i/ B_0 %

absorbance maximum binding

7. Results

Since the samples have been diluted prior to assay, the concentrations in ng/ml read from the standard curve must be multiplied by the respective dilution factor to obtain the effective kanamycin concentration in samples expressed in ppb (ng/ml or ng/g). The dilution factors are 50 for milk, 40 for meat, 10 for liquid medicine.

8. Cautions

1) Each reagent is optimized for use in the UC Biodevices Kanamycin Plate Kit. Do not substitute regents from any other manufacturer.
2) Avoid contacting the reagents (particularly standard solutions) with the skin (wear gloves)
3) If ambient temperature is lower than 20℃ or the temperature of the reagents and standard solutions is less than the ambient temperature (e.g., 20- 25℃） when used, the testing results may have negative bias (lower than it should be).
4) If it takes too long time to dry the washed microtiter during the washing steps, the results may demonstrate nonlinear calibration plot and poor repeatability, thus once the microtiter is dried after the washing steps, the following step should be immediately implemented to prevent the undesirable occurrence.
5) The standard solutions and chromogenic solution contain light-sensitive substances, thus the solutions should not be exposed under direct light.
6) To gain excellent results, any solutions should be thoroughly mixed before added on microtiter and the microtiter should be completely cleaned in between each step.
7) The stop solution is a very strong acidic solution, so users should avoid the solution directly contacting with skin.
8) Neither use expired reagents, nor dilutes reagents.
9) Do not interchange individual reagents between kits with different lot numbers.
10) The kit should be stored at 2-8℃ but not be frozen. Place any unused microwells to their original

foil bag and reseal them together with the desiccant provided.

If there is difficulty to get great results by following the manual carefully, the matrix for the testing samples may be the cause. Please contact us and we can provide helps to develop a new assay kit or kits for your specific applications.

9. Contact Information

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