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User Manual

Disclaimer: Products are intended for research use only

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SHENTEK

Kanamycin ELISA Kit

User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1401402
Version: A/1
For Research Use Only

Biofargo, Inc.

■ Product Name

Kanamycin ELISA Kit

■ Package

96 tests/Kit

■ Intended Use

This kit is suitable for the quantitation of kanamycin residues in plasmid DNA, including cell and gene therapy products.

The kit is for RESEARCH USE ONLY and not intended for clinical use.

■ Product Description

This ELISA kit utilizes an "indirect-competitive" enzyme immunoassay. The microplate wells are coated with kanamycin antigen, that competes with Kanamycin in a sample for anti-kanamycin monoclonal antibody. An enzyme-tagged secondary antibody targets the primary monoclonal antibody that is complexed with the kanamycin coated on the plate wells. After an addition of TMB substrate, the resulting color intensity at 450nm has an inverse relationship with the Kanamycin residue concentration in the sample. The total amount of kanamycin in the sample is obtained by comparison with a standard curve, and adjusted by the sample dilution factor.

For the detection of residual kanamycin in other biologics samples, sample suitability test is recommended to assess the effects of matrix interference.

■ Kit Contents

Table 1. Kit Components

Reagent	Part No.	Quantity	Storage
Kanamycin Standard	PNB001	2 bottles	2-8℃, protect from light
Kanamycin Standard Reconstitution Diluent	PNC001	2×1.5 mL	2-8℃
Kanamycin Standard Solution Diluent	PNE001	1×20 mL	2-8℃
100×Kanamycin Primary Antibody	PNG001	1×60 μL	2-8℃
100×Enzyme-conjugated Secondary Antibody	PNH001	1×120 μL	2-8℃, protect from light
Kanamycin-coated Plate	PNA001	8 well×12 strips	2-8℃, protect from light
10×Wash Buffer	PNJ001	2×25 mL	2-8℃
Chromogenic Substrate for Kanamycin ELISA	PND001	1×12 mL	2-8℃, protect from light
Stop Solution	PNI001	1×12 mL	2-8℃
Sealing Film	PNK001	2 pieces	2-8℃

Note: Room temperature refers to 25 ± 3℃.

■ Storage Conditions

The entire kit can be stored at appropriate conditions for up to 12 months. Please check the expiration date on the kit labeling.

■ Materials Required But Not Provided

- Deionized water
- Sterile graduated pipettes, 10mL
- RNase/DNase-free sterile centrifuge tubes,1.5mL, 50mL

- Single-channel pipettes,10μL, 200μL, 1000μL
- Multi-channel pipette, 200μL (or 300μL)
- RNase/DNase-free tips, 1000μL, 100μL, 10μL

■ Equipment

- Microplate reader (450 nm)
- Incubator (25℃±3℃)
- Microplate thermoshaker
- Centrifuge

■ Assay Performance

Detection limit: < 0.5 ng/mL;

Detection range: 1 ng/mL-16 ng/mL;

Accuracy: Spike recovery is 80%-120%;

Precision: The CV of the ELISA kit is all less than 20%

Cross-reaction rate: See table 2.

Table 2. Cross-reaction Rate

Antibiotics	Cross-reaction Rate
Kanamycin	100%
Streptomycin	<1%
Ampicillin	<1%
Chloramphenicol	<1%
Tetracycline	<1%

■ Workflow

1. Preparation

(1) Equilibration

- Allow the kit to equilibrate at room temperature for 30 minutes before use. Take out the required number of strips to be used and return the

remaining strips and the desiccant to the original foil bag as soon as possible. Then store at 2-8°C for up to one month.

- Mix all reagents thoroughly before use. Avoid foaming. Spin 100×Kanamycin Primary Antibody and 100×Enzyme-conjugated Secondary Antibody tubes for 10 seconds to bring down all components to the bottom of tubes.

(2) Preparation of Reagents

- Preparation of wash solution: Dilute the 10×Wash Buffer (PNJ001) with deionized water at a volume ratio of 1:9 to prepare the wash solution (for example, add 45 mL deionized water to 5 mL of 10×Wash Buffer in a 50 -mL centrifuge tube).

  Note: If crystals have formed in the 10×Wash Buffer, warm it up in a 37℃ water bath and mix gently until the crystals have completely dissolved.

  Note: This solution can be stored at 2-8℃ for 30 days.

- Preparation of kanamycin stock standard solution: Follow in accordance with the content label on the Kanamycin Standard bottle (PNB001), add the corresponding volume of Standard Reconstitution Diluent (PNC001) and mix well, to prepare 1 μg/mL kanamycin stock standard solution.

  Note: This kanamycin stock standard solution can be stored at 2-8℃ for up to 30 days and should be protected from light.

(3) Preparation of Calibration Standard Solutions

- Prepare a dilution series of kanamycin Standard Solutions in the concentration range of 64 ng/mL, 32 ng/mL, 16 ng/mL, 8 ng/mL, 4 ng/mL, 2 ng/mL, 1 ng/mL, 0.5 ng/mL by diluting the Kanamycin stock standard solution in Standard Solution Diluent (PNE001). Please follow the below steps:

1. Take 8 new 1.5 mL centrifuge tubes and label ST0, ST1, ST2, ST3, ST4, ST5, ST6, ST7 respectively.

2. Add 936 μL Kanamycin Standard Solution Diluent to the ST0 tube, and add 400 μL of Standard Solution Diluent to each of the ST1-ST7 tubes.
3. Add 64μL of the Kanamycin stock standard solution to the ST0 tube, mix well and quickly centrifuge for 5 seconds, repeat 3 times to mix well.
4. Prepare a series of kanamycin standard solutions according to Table 3, and follow the serial dilution procedure in Step 3 above.

Table 3. Preparation of Kanamycin Standard Solutions

Standard	**Dilution**	**Conc.** (ng/mL)
ST0	64 μL kanamycin stock standard solution (1 μg/mL) +936 μL Kanamycin Standard Solution Diluent	64
ST1	400 μL ST0 + 400 μL Kanamycin Standard Solution Diluent	32
ST2	400 μL ST1 + 400 μL Kanamycin Standard Solution Diluent	16
ST3	400 μL ST2 + 400 μL Kanamycin Standard Solution Diluent	8
ST4	400 μL ST3 + 400 μL Kanamycin Standard Solution Diluent	4
ST5	400 μL ST4 + 400 μL Kanamycin Standard Solution Diluent	2
ST6	400 μL ST5 + 400 μL Kanamycin Standard Solution Diluent	1
ST7	400 μL ST6 + 400 μL Kanamycin Standard Solution Diluent	0.5
NCS	Kanamycin Standard Solution Diluent	0

The standard curve range is between ST1 and ST7.

■ Sample preparation

1. Preparation of the sample solution: centrifuge liquid sample at 5000 rpm for 5 min, and recover the supernatant for assay.

Note: Dilute the supernatant with Standard Solution Diluent as necessary.

Note: In order to assess the effects of matrix interference, spiked samples can be prepared, for example, to add a known amount of kanamycin standard solution to the test sample or diluted test sample for a spiking level of 4 ng/mL in the sample.

2. Standard/sample solution addition: number every microplate well position for the prepared sample solution, Kanamycin standard solutions (ST1~ST7) and Kanamycin standard solution diluent (NCS). All standards and samples should be run in triplicate and record the standards and samples positions. Please carefully add 50μL/well of standard and sample solution in the corresponding wells (Single-channel pipette is recommended).
3. Preparation of 1×kanamycin primary antibody working solution: dilute 100×Kanamycin Primary Antibody (PNG001) with wash solution to prepare 1×kanamycin primary antibody working solution, which should be appropriately more than the necessary volume for experiment (calculation based on 50μL/well).
4. 1×kanamycin primary antibody working solution addition: add 50μL/well of 1× kanamycin primary antibody working solution to each well (Single-channel pipette is recommended). Cover the plate with sealer and oscillate the plate for 30s at 200rpm to mix thoroughly, then incubate at 25℃ for 60 min.
5. Preparation of 1×Enzyme-conjuaged secondary antibody working solution: dilute 100× Enzyme-conjugated secondary antibody solution (PNH001) with wash solution (see “Reagent preparation before test” section) to prepare 1× enzyme-conjugated secondary antibody working solution, which should be 200μL (2-well volume) more than the necessary volume for experiment (calculation based on 100μL/well).
6. Plate washing: uncover the sealer carefully, Wash the plate with 350 μL of 1×Wash Buffer per well for 30 seconds. Wipe off any liquid from the bottom outside of the plate. Repeat washing for 4 times. Do not allow the wells to be completely dry before adding the substrate.
7. 1×Enzyme-conjuaged secondary antibody working solution addition: Add 100μL 1×Enzyme-conjugated secondary antibody working solution to each well (Either single-channel or multi-channel pipette can be used). Cover the plate with sealer and oscillate for 30s at 200 rpm to mix thoroughly, then incubate at 25℃ for 60 min. Protect from light.

8. Plate washing: Same as step 6.
9. Color development: Add 100μL/well of chromogenic substrate (PND001) to each well, gently oscillate the plate to mix thoroughly and incubate at 25℃ for 15 min. Protect from light.
10. OD measurement: add 100μL/well of stop solution (PNI001) to each well, gently oscillate the plate to mix thoroughly. Then measure the absorbance at 450nm and read the result in 10 min±2min right after addition of stop solution. Protect from light.

■ Data analysis

There are two methods to judge the results, the first one is a rough estimate and the second is a quantitative determination. Note that the OD value of the sample is inversely correlated with the kanamycin concentration in the sample.

1. The kanamycin concentration in the sample can be derived by comparing the average OD value of the sample with the standard curve. For example, if the absorbance value of the sample is between those of standards 2 ng/mL and 4 ng/mL. The kanamycin concentration in the sample is 2 ng/mL~4 ng/mL, multiplied by its corresponding dilution factor, the actual concentration of kanamycin residues in the sample can be obtained.

2. Quantitative determination

(1) Absorbance (%) is the mean values of the absorbance obtained for the standards or samples divided by the mean absorbance of the 0 value and multiplied by 100%, that is:

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

B — Average absorbance of the standard or sample solution

B_0 — Average absorbance of 0 value solution

(2) Drawing and calculation of standard curve

Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the concentration (ng/mL) of kanamycin standard solution

on the x-axis to fit 4 parameter logistic regression model. The standard curve ranges from ST1 to ST7, and the value of 0 is not included in the standard curve.

4-Parameter Logistic mode is: $y=(a-d)/[1+(x/c)^b]+d$

The actual kanamycin concentration in the sample is calculated from the standard curve with the absorbance percentage of the sample, and multiplied by its corresponding dilution factor.

■ Additional Information

1. The optimum reaction temperature is 25℃. Temperature too high or too low will lead to variations in absorbance value and assay sensitivity. The microplate incubation temperature should be at 25±3℃ to maintain robustness of the assay. Temperature below 20℃ will lead to a decrease in absorbance values and should be avoided.
2. The reagents should be mixed well before use, and returned to a refrigerator (2 - 8℃) immediately after use.
3. The samples, calibration standards, 1x kanamycin antibody working solution should be dispensed accurately. Single-channel pipettes are recommended.
4. Reproducibility in ELISA tests depend on the consistency of plate washing, and proper washing is the key step in the ELISA assay procedure. When pipetting the wash solution into the microplate, the pipette tips should be handled carefully to avoid touching any liquid in the wells. In case of any contact, the tips should be replaced. Do not allow microwells to dry between steps to avoid bad linear standard curve and unsuccessful repeatability, etc. Perform the next step immediately after decanting, and make sure the reagents are prepared in advance and ready to use.
5. During all incubation steps, the plates should be sealed properly (except the color development step) and protected from light.
6. Stop solution is caustic, please wear goggles and avoid contact with skin and eyes.
7. Do not use the kits past the expiration date. Do not mix reagents from different lots.
8. Storage conditions

   Store the kit at 2 - 8℃. Do not freeze any kit components.

Return any unused strips to the original foil bag immediately with the desiccant to avoid moisture, and seal tightly for up to 30 days.

Kanamycin standard, secondary antibody and chromogenic substrate are light sensitive and should be protected from light.

The kanamycin standard solutions, and enzyme-conjugated secondary antibody working solutions should be prepared on the same day of use, and should not be stored for re-use. The working solutions should be prepared in an appropriate volume required for the experiment, and for immediate use.

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Support & Contact

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