Kanamycin ELISA Kit

User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1401402
Version: A/2
For Research Use Only. Not for diagnostic or therapeutic use.

Huzhou Shenke Biotechnology Co., Ltd

■ 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 product described herein is for research use only and is not intended for diagnostic or therapeutic 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 (3,3',5,5'-tetramethylbenzidine) substrate, the resulting color intensity at 450 nm 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.

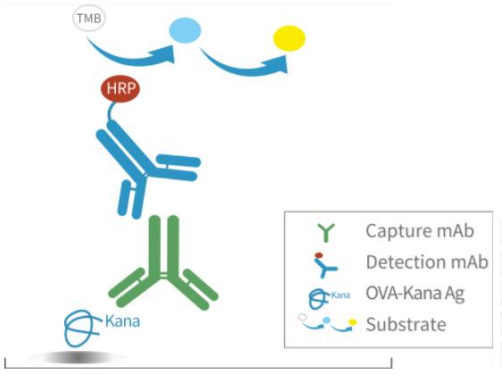


Figure 1. Schematic diagram

■ 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

Store the kit at 2-8℃. Use within the expiration date labeled upon the kit package.

The opened components should be stored as follows.

Table 2. Recommended storage conditions for opened components

Component	Stability
Kanamycin-coated Plate	Store in the bag with desiccant at 2-8℃ for up to 30 days.
Kanamycin Standard	Store at 2-8℃ for up to 30 days

■ Materials Required (Not Provided in the Kit)

- Sterile centrifuge tubes for dilution
- Absorbent paper for plate drying
- Pipette Tips: 1000 μL, 100 μL, and 10 μL
- Multi-channel reagent reservoirs (50 mL)

■ Equipment

- Microplate reader capable of measuring absorbance at 450 nm.
- Single or multi-channel pipettes
- Microplate thermoshaker
- Incubator (optional)
- Plate washer (optional)

■ Workflow

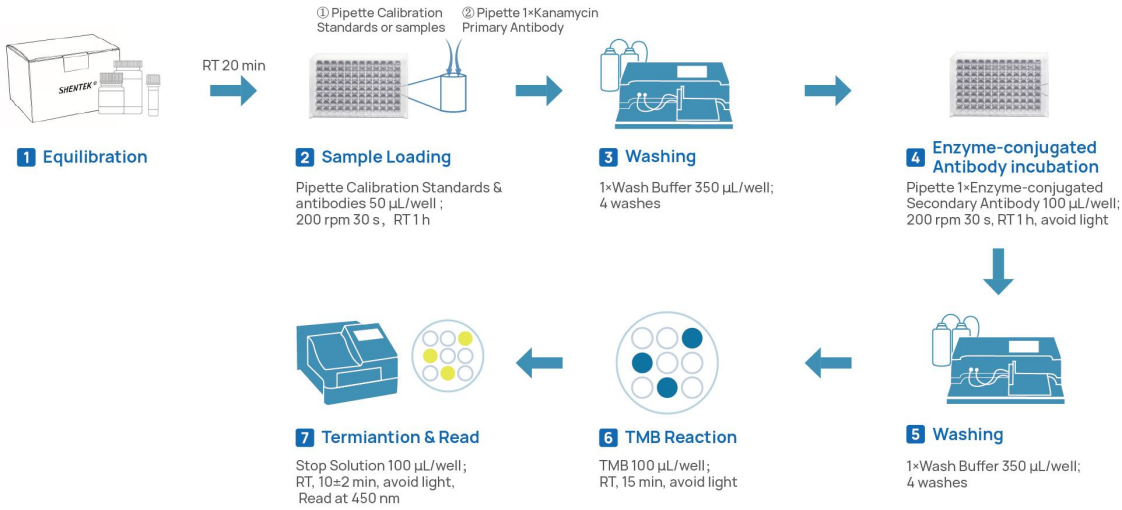


Figure 2. Procedure Flowchart

1. Preparation

(1) Equilibration

- Allow the kit to equilibrate at room temperature for 20 minutes before use. Take the appropriate amount of strips to a strip holder according to the experiment design and store the remaining strips in the bag with desiccant at 2-8℃.
- 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

- Kanamycin Standard solution: Accordance with the content label on the Kanamycin Standard (PNB001) bottle, add the corresponding volume of Kanamycin Standard Reconstitution Diluent (PNC001) and mix well, to prepare 1 µg/mL kanamycin stock standard solution. Save the remaining solution under the recommended condition.

$$\text{Kanamycin Standard Reconstitution Diluent (PNC001) (mL)} = \frac{\text{Kanamycin Standard (PNB001) (μg)}}{1\ \mu g/mL}$$

For example, if the label indicates that a bottle of Kanamycin Standard (PNB001) contains 0.84 μg, adding 0.84 mL (840 μL) of Kanamycin Standard Reconstitution Diluent (PNC001) yields a kanamycin stock standard solution at 1 μg/mL.

Note: *If two or more vials of calibration standards are applied, combined all after reconstituted, and mix gently before use. Do not use any other volumes of Kanamycin Standard Reconstitution Diluent (PNC001) to dissolve the Calibration Standard.*

- 1×Wash Buffer: Dilute 1 volume of Wash Buffer with 9 volumes of ultra-pure water. For example, add 25 mL 10×Wash Buffer (PNJ001) to 225 mL of ultra-pure water to make 250 mL of 1×Wash Buffer. Mix well before use.

  Note: *If the 10×Wash Buffer (PNJ001) or Kanamycin Standard Solution Diluent (PNE001) is cloudy or contains precipitates, heat at 37 ℃ until it clears.*

- 1×Kanamycin Primary Antibody: Prepare the 1×Kanamycin Primary Antibody by diluting the 100×Kanamycin Primary Antibody (PNG001) with 1×Wash Buffer in a sterile centrifuge tube. Gently mix the solution and use it immediately.

- 1×Enzyme-conjugated Secondary Antibody: Prepare the 1×Enzyme-conjugated Secondary Antibody by diluting the 100×Enzyme-conjugated Secondary Antibody (PNH001) with 1×Wash Buffer in a sterile centrifuge tube. Gently mix the solution and use it immediately.

(3) Preparation of Calibration Standard Solutions

- Prepare Kanamycin Calibration Standard solutions as indicated in Figure 3 and Table 3.

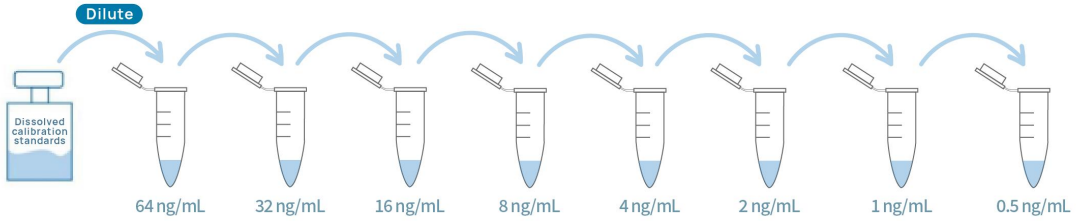


Figure 3. Graphic scheme of Kanamycin Calibration Standard solutions

Table 3. Preparation of Kanamycin Standard Solutions

Tubes	Dilution procedure	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

Note: *The standard curve range is between ST1 and ST7.*

(4) Sample preparation

- Preparation of the sample solution: centrifuge liquid sample at 5000 rpm for 5 minutes, and recover the supernatant for assay. Dilute the supernatant with Kanamycin Standard Solution Diluent as necessary.
- Conduct sample stability studies to prevent degradation or denaturation during the experiment. Long-term storage at -70 °C is recommended to avoid degradation and avoid repeated freeze-thaw cycles.
- 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. Assay Experiment

(1) Sample Loading & Incubation

- Pipette 50 μL of Calibration Standard, controls and samples into the corresponding wells as indicated earlier. Avoid foaming bubbles during pipetting. It is recommended to prepare 2-3 parallels for each concentration.
- Pipette 50 μL of 1×Kanamycin Primary Antibody solution into each designated well according to the experiment design.
- Cover the plate with sealer and oscillate the plate for 30 seconds at 200 rpm to mix thoroughly, then incubate at 25℃ for 60 minutes.

Table 4. Example of the microplate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	NCS	NCS	NCS		S1	S1	S1					
B	ST7	ST7	ST7		S2	S2	S2					
C	ST6	ST6	ST6		S3	S3	S3					
D	ST5	ST5	ST5		S1+SRC	S1+SRC	S1+SRC					
E	ST4	ST4	ST4		S2+SRC	S2+SRC	S2+SRC					
F	ST3	ST3	ST3		S3+SRC	S3+SRC	S3+SRC					
G	ST2	ST2	ST2									
H	ST1	ST1	ST1									

- ✧ “ST1-ST7” indicate 7 concentration gradients, “NCS” as negative control, “S1-S3” as test samples, and “S1 SRC-S3 SRC” as the spiked recovery controls for each sample.
- ✧ The number of replicates and the spiked samples can be determined by method validation.

(2) Enzyme-conjugated Antibody Reagent Incubation

- Wash the plate with 350 μL of 1×Wash Buffer per well and soak 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.

- Pipette 100 μL of 1×Enzyme-conjugated Secondary Antibody solution into each designated well according to the experiment design.
- Cover the plate with sealer and oscillate the plate for 30 seconds at 200 rpm to mix thoroughly, then incubate at 25℃ for 60 minutes. Protect from light.

(3) Substrate Incubation

- Equilibrate the Chromogenic Substrate for Kanamycin ELISA (PND001) for 20 minutes at room temperature.
- Wash the plate with 350 μL of 1×Wash Buffer per well and soak 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.
- Add 100 μL of Chromogenic Substrate for Kanamycin ELISA (PND001) into the wells, and incubate at room temperature for 15 minutes, and protect from light.

  Note: *Do not use Sealing Film (PNK001) during this step.*

(4) Termination

- Add 100 μL of Stop Solution (PNI001) into each well.

  Note: *The order of adding Stop Solution (PNI001) should be the same as the order of adding the Chromogenic Substrate for Kanamycin ELISA (PND001). While adding samples, suspend the tips above the liquid to prevent contact with the solution in the wells and minimize the risk of bubble formation.*
- Incubate at room temperature for 10±2 minutes, protect from light.

(5) Reading

- Read absorbance at 450 nm.

3. Calculation and 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.
- Determine the kanamycin concentration by interpolating the sample's average OD value against the standard curve. For example, if the OD falls between the 2 ng/mL and 4 ng/mL standards, interpolate to obtain the exact concentration within this range. Multiply this interpolated value by the sample dilution factor to calculate the final kanamycin residue concentration in the original sample.
- 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.

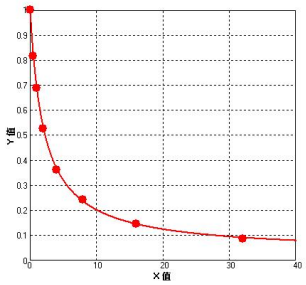
■ Assay Performance

- Linearity & Range: 1 ng/mL-16 ng/mL, $R^2 \geq 0.990$
- DL: < 0.5 ng/mL
- Accuracy: Spike recovery is 80%-120%
- Precision: The CV of the kit is all less than 20%
- Specificity:

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

- Typical calibration curve and data:

STD (ng/mL)	Mean ($OD_{450\ nm}$)	CV (%)
32	0.087	1.1%
16	0.146	3.5%
8	0.243	0.1%
4	0.362	1.1%
2	0.527	4.3%
1	0.689	2.1%
0.5	0.816	1.5%
0	1.000	1.1%



4-PL: $Y = \frac{A-D}{1+(\frac{X}{C})^B}+D$

A = 1.00111
B = 0.98902
C = 2.12418
D = 0.02886
R^2 = 0.99984

■ Additional Information

- ✧ This kit is intended for use by qualified technicians only.
- ✧ Use sterile disposable tips, tubes and reservoirs, etc. separately. It is recommended to wipe with 75% ethanol before and after each use. Follow the specified pipetting procedure carefully.
- ✧ Users should validate the assay before testing their samples.
- ✧ Dilution should be gentle and thorough to avoid excessive foaming.
- ✧ Stop Solution (PNI001) is caustic. Avoid direct contact with eyes, skin, and clothing.
- ✧ Do not mix the kit reagents with different lot numbers.
- ✧ Use fresh sterile water or ultra-pure water, and ensure the water temperature does not exceed 37℃.
- ✧ Seal or cover the microplate immediately after sample loading to avoid liquid evaporation.
- ✧ Avoid drying the wells before substrate incubation.
- ✧ Store unused microtiter strips in a sealed bag with desiccant to prevent contamination.
- ✧ Centrifuge 100×Kanamycin Primary Antibody (PNG001), 100×Enzyme-conjugated Secondary Antibody (PNH001) before use avoid any loss of the reagent.
- ✧ Accurately pipetting or sampling for dilution of standards and samples, for example, minimum volume of 5 μL is recommended.
- ✧ Kanamycin Standard solutions, 1×Kanamycin Primary Antibody and 1×Enzyme-conjugated Secondary Antibody are recommended for single use due to instability issue. Prepare freshly before each experiment.
- ✧ Chromogenic Substrate for Kanamycin ELISA (PND001) should be colorless. If not, discard it and contact us for assistance.
- ✧ Pipette carefully to avoid any bubbles, and gently shake the plate for thorough mixing. Bubbles can influence optical density values and detection results.
- ✧ Reading should be completed within 30 minutes after termination.
- ✧ Avoid the samples containing sodium azide (NaN_3), which will deactivate the HRP and lead to the underestimation of samples levels.

■ References

- USP<1103> Immunological Test Methods Enzyme-Linked Immunosorbent Assay (Elisa)
- ChP <9101> Guidance for Analytical Procedures Validation

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Support & Contact

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