Ver202505

Instructions for Use of Kanamycin ELISA Detection Kit(2G)

Cat.No. HG-KA002

Contents

1. Product Description

This kit uses indirect competitive ELISA to detect trace residues of kanamycin in samples.The microplate is pre-coated with a conjugate antigen. Kanamycin residues in the sample compete with the pre-coated conjugate antigen on the microplate for binding to anti-kanamycin antibodies. After adding the enzyme-labeled secondary antibody, the reaction is visualized by adding TMB substrate. The absorbance (OD value) is measured at 450 nm/630 nm using a microplate reader, and the percentage absorbance is calculated. The kanamycin concentration in the sample is inversely proportional to the percentage absorbance.

Detection Range :0.128 ng/mL 12.5 ng/mL

2. Applications

This kit is specifically optimized for the detection of kanamycin residues in recombinant plasmid samples, and is also applicable for the detection of kanamycin residues in other protein samples.

3. Kit Specifications

Product Name	Cat. No.	Specifications
Kanamycin ELISA Detection Kit(2G)	HG-KA002	96T

4. Kit Components

Components	Specifications	Storage Conditions
Coated Plate	8 wells ×12 strips	2 8
Kan Standard 50 ng/mL	1 mL×1 vial	
Anti-Kan Biotin	7 mL×1vial	
Streptavidin HRP	12 mL×1 vial	
Sample Diluent Buffer	30 mL×1 vial	

Components	Specifications	Storage Conditions
20×Wash Buffer	30 mL×1 vial	
Color Reagent A	8 mL×1 vial	
Color Reagent B	8 mL×1 vial	
Stop Solution	15 mL×1 vial	
Sealing Membrane	5 pieces	
Manual	1 copy	

5. Storage Conditions and Shelf Life

This kit should be stored at 2 8°C and protected from light. The kit has a shelf life of 12 months from the date of manufacture. For unused kit after opening , store at 2 8°C and protected from light.

6. Definitions/Terminology

- OD: Optical Density
- CV: Coefficient of Variation, defined as the ratio of the standard deviation to the mean.
- ERC Extraction Recovery Control

7. Required Reagents, Consumables & Equipment

- Microplate reader
- Microplate incubator/shaker (Provide incubation temperature)
- Vortex mixer
- Deionized water
- Absorbent paper
- Micropipettes & tips

8. Operation Procedure

8.1. Assay Workflow Diagram

Total Duration: About 1 Hour and 10 Minutes

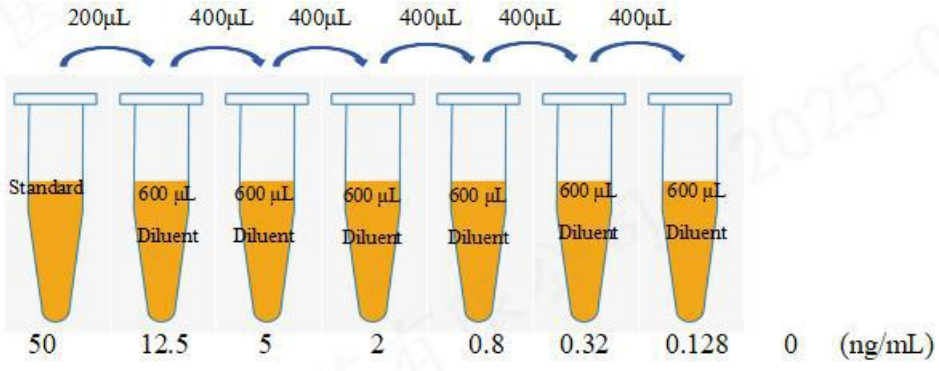
8.2. Preparation

8.2.1. Remove the kit from storage at 2 8 °C and allow all reagents to equilibrate to room temperature (at least 30 minutes) prior to use. Ensure that sufficient reagents are available for the entire experiment. Record the opening date on the kit when first opened, and always use the earliest opened kit first. Kits from the same lot number may be used interchangeably; do not mix components from different lot numbers. Prepare only the required number of ELISA strips for the current test. Return any unused strips to the foil sealed bag, and store at 2 8 °C for future use.

8.3. Preparation of 1× Wash Buffer: Dilute the 20× Wash Buffer concentrate 20-fold with deionized water. If crystals are observed in the 20× Wash Buffer, warm the solution to room temperature or place it in a 37 °C water bath with gentle shaking until fully dissolved before dilution.

8.4. Preparation of Substrate Solution: Mix equal volumes of Substrate Solution A and Substrate Solution B thoroughly and protect from light. (Note: The substrate solution should be freshly prepared approximately 10 minutes before use. Do not use the mixture if it has already turned blue.

8.5. Preparation of Reference Standard: Add 200 µL of the standard solution (original concentration: 50 ng/mL) to 600 µL of sample diluent to obtain a 12.5 ng/mL diluted standard solution. Perform 2.5-fold serial dilutions to prepare a standard series. (Fresh standard solutions should be prepared before each experiment.) See the diagram below for the dilution steps.



8.6. Preparation of Test Sample Solution: Add 200 µL of the test sample to 200 µL of sample diluent and mix thoroughly.

8.7. Preparation of Extraction Recovery Control: Add 100 µL of the test sample solution to 100 µL of the 2 ng/mL standard solution and mix well.

8.8. Sample Incubation: Add 50 µL of standard solution, blank control (sample diluent), or test sample to each designated well. Then add 50 µL of Detection Antibody to each well. Cover the plate with sealing film and incubate at 25 °C in the dark for 30 minutes.
(Note: Standard solution must be added before the antibody to prevent immediate reaction with the coated antigen. Incomplete sealing or failure to seal the plate may result in evaporation and affect test accuracy. Avoid light exposure during incubation.)

8.9. Plate Washing: After incubation, carefully remove the sealing film and discard the well contents. Wash the plate 3 times with 1× Wash Buffer (250 µL/well), remove residual liquid by gently tapping the plate on absorbent papers.
(Note: If washing manually, dispense wash buffer while keeping the pipette tip above the well surface to avoid contact. Let the buffer stand for 30 seconds with gentle agitation before discarding. Use clean, unused portions of absorbent papers to remove residual substances from each well.)

8.10. Enzyme-Conjugated Secondary Antibody Incubation: Add 100 µL of enzyme-conjugated secondary antibody to each well. Cover the plate and incubate at 25 °C for 30 minutes, protected from light.

8.11. Plate Washing: After incubation, carefully remove the sealing film, discard the well liquid, and wash the plate 3 times with 1× Wash Buffer (250 µL/well). Remove residual liquid by gently tapping the plate on absorbent papers.
(Note: Same precautions as in section 8.9 apply.)

8.12. Color Development Add 100 µL of freshly prepared substrate solution to each well. Cover the plate and incubate at 25 °C in the dark for 10 15 minutes.

8.13. Reaction Termination Add 100 µL of Stop Solution to each well. Once color is evenly developed, the plate can be read.
(Note: It is recommended to set a 5 10 second shaking step in the microplate reader program.)

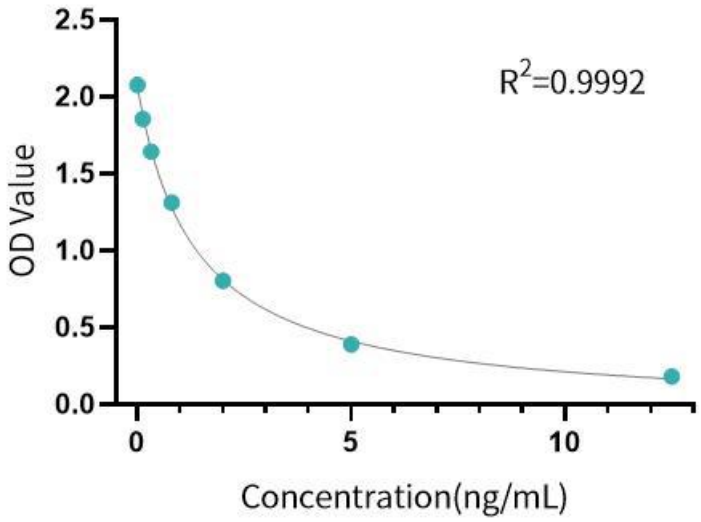
8.14. OD Reading Within 20 minutes of adding Stop Solution, measure absorbance at 450 nm with a reference wavelength of 630 nm using a microplate reader.

9. Result Analysis

9.1. Standard Curve Fitting: Plot the absorbance of the standards on the y-axis and their concentrations on the x-axis. Use a four-parameter logistic regression equation to fit the standard curve.

9.2. Standard Curve Fitting Example Figure

Concentration of standards(ng/mL)	OD Value	Detection value (ng/mL)	Rate of recovery (%)
0	2.07670	<Min	/
0.128	1.85530	0.14166	111
0.32	1.64210	0.33076	103
0.8	1.31140	0.74654	93
2	0.80240	2.03440	102
5	0.38890	5.31858	106
12.5	0.18000	11.57690	93



9.3. Result Calculation

9.3.1. Calculation of Testing Example Residual Concentration (ng/mL)

$$\text{Teting Sample Kanamycin Residual Concentration} \quad ng/mL$$
$$= \text{Dilution factor} \times \text{Fitting Concentration of Testing Sample}$$

Example: if Dilution factor is 2

$$\text{Teting Sample Kanamycin Residual Concentration} \quad ng/mL$$
$$= 2 \times \text{Fitting Concentration of Testing Sample}$$

9.3.2. ERC sample Recovery Rate (%) Calculation:

$$ERC\ Recovery\ Rate\% = \frac{(ERC\ Sample\ Concentration \times Total\ Volume) - (Test\ Sample\ Concentration \times Sample\ volume)}{ERC\ Sample\ Theoretical\ Concentration \times ERC\ Sample\ Volume} \times 100\%$$

10. Precautions

10.1. The optimal reaction temperature for this kit is 25 °C. Deviations from this may affect absorbance and sensitivity.

10.2. All components must be brought to room temperature (18 25 °C) before use.

10.3. Mix all components thoroughly before use. Briefly centrifuge standards for 5 seconds to collect liquid from tube walls and caps. Return all reagents to 2 8 °C immediately after use.

10.4. Use the kit within its expiration date. Prepare a new standard curve for each test. Mixing reagents from different lot numbers is not recommended.

10.5. When pipetting into wells, avoid touching the bottom to protect the coated layer. Change pipette tips and reservoirs between different samples or steps to prevent cross-contamination.

10.6. Plate After washing, tapping ELISA strips carefully to avoid dislodging. Do not reuse sealing film.

10.7. Formation of dark precipitates during color development at high concentrations is normal and does not affect the final reading if minimal.

10.8. Ensure correct detection wavelength and curve-fitting model are selected during data analysis.

10.9. Optimal results are obtained only when following the instructions strictly and using only the reagents provided in the kit.

10.10. Variability in results may arise from multiple sources including operator technique, pipetting accuracy, plate washing method, incubation time and temperature, and reagent storage conditions.

10.11. The manufacturer is only responsible for the quality of the kit itself and not for sample loss resulting from its use. Users should reserve sufficient sample volume prior to testing.

10.12. This kit is intended for research use only and not for clinical diagnosis.

11. Troubleshooting

Seq. No.	Problem Description	Possible causes	Countermeasures
1	Standard Curve Gradient Issues	Inaccurate pipetting or liquid transfer	Check pipettes and pipette tips
		Incomplete microplate washing	Ensure proper wash cycles and sufficient wash buffer volume per well
2	Weak or No Color Development	Insufficient incubation time	Ensure adequate incubation duration
		Incorrect reaction temperature	Use recommended incubation temperature
		Inadequate reagent volume or missed addition	Verify pipetting procedures to ensure all reagents are added sequentially in correct volumes
		Improper substrate preparation	Prepare substrate solution by mixing Components A and B in 1:1 ratio immediately before use (10 min prior to detection), protected from light
3	Low OD Readings	Incorrect microplate reader settings	Verify wavelength and filter settings on microplate reader
			Preheat instrument prior to readings as recommended
4	High Coefficient of Variation (CV)	Pipetting inconsistencies	Perform retrospective analysis or validation tests to ensure accurate and uniform reagent dispensing
		Pipette calibration issues	Conduct regular calibration and performance verification of pipetting equipment
		Well contamination or	Inspect wells for debris before

		bubbles	addition and bubbles after dispensing
		Microplate bottom contamination	Examine plate bottom for residual liquids or fingerprints
		Improper plate sealing during incubation	Seal plate completely using adhesive plate sealers
5	High Background Value	Incomplete plate washing	Follow manufacturer's recommended washing protocol
			For automated washers: Verify all fluid ports are unobstructed
			For manual washing: Increase wash cycles as needed
		Contaminated common reagents	Replace with fresh, uncontaminated reagents
		Contaminated shared equipment	Use dedicated pipettes with sterile filtered tips
		Cross-contamination from shared workspaces	Establish physically separated work zones for different procedures
		Incorrect reagent preparation	Prepare fresh solutions using proper dilution factors
6	Reduced Sensitivity	Improper kit storage	Store all kit components according to manufacturer specifications

12. References

- Pharmacopoeia of the People s Republic of China (2020 Edition)
- I ICH Guidelines Q6B: Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products

13. Contact Information

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- After-sales E-Mail Address: technical@hillgene.com
- Website: https://www.hillgene.com

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Appendix 1: Safety Precautions

- General Instructions

Failure to use this product in accordance with the instructions provided in this manual may result in personal injury or damage to instruments or equipment. Ensure that personnel using this product have received appropriate training in general laboratory safety practices and have reviewed the safety information provided herein.

(1) Before operating any instrument or equipment, read and understand all safety information provided in the user documentation from the respective manufacturer.
(2) Prior to handling any chemicals, read and understand all applicable Safety Data Sheets (SDS), and wear suitable personal protective equipment (PPE), including gloves, protective clothing, and safety goggles.

- Biological Hazards

(1) Biological samples, including tissues, bodily fluids, infectious agents, and blood from humans or other animals, may pose a risk of transmitting infectious diseases. All procedures must be conducted in facilities equipped with appropriate safety equipment, such as certified biological safety cabinets. Additional safety measures include the use of personal protective equipment (PPE), such as gloves, lab coats, coveralls, shoe covers, boots, respirators, face shields, safety glasses, or goggles.
(2) Individuals working with potentially biohazardous materials must receive training in accordance with local regulations and institutional or corporate requirements prior to handling such materials.

- Hazardous Waste (from Instruments)

Waste generated by the instruments may present potential hazards. Follow the guidelines described under Biological Hazards to handle such waste safely.

Appendix 2: Related products For more products, please consult:https://www.hillgene.com

Category	Product name	Cat. No.
Plasmid Detection	E.coli Residual DNA Detection Kit (qPCR)	HG-ED001
	E.coli Residual DNA Fragment Analysis Detection Kit (qPCR)	HG-EF001
	E.coli Residual Total RNA Sample Preprocessing Kit	HG-CL300
	E.coli Residual Total RNA Detection Kit (RT-PCR)	HG-ER001
	E.coli HCP ELISA Detection Kit(2G)	HG-HCP002-2G