EZElisa™ Kanamycin Residual ELISA Kit

Cat #: A-QEK007

Size: 96 Tests

Storage: 2-8 °C in dark (The production date and shelf life are shown on label of the kit.)

Product information

Product description

Kanamycin is an aminoglycoside antibiotic used in the treatment of animal diseases, strain screening, and for the preparation of cell and gene therapy drug materials. Due to its neurotoxicity, nephrotoxicity, and ototoxicity, the presence of kanamycin residues in animal food and biopharmaceuticals can affect human health and even cause allergic reactions. Therefore, both European and American countries, as well as China, have regulations limiting its use. This kit is designed for the detection of kanamycin residues in cell and gene therapy drug materials, intermediates, semi-finished products, and finished products. For the testing of other biological product samples, users are advised to conduct suitability validation to eliminate interference from the matrix.

Principles

This kit utilizes an indirect competitive ELISA method to detect trace levels of kanamycin residues in samples. The microplate is pre-coated with a conjugated antigen, and the residual kanamycin in the sample competes with the conjugated antigen on the microplate strip for the anti-kanamycin antibody. After the addition of the enzyme-labeled secondary antibody and subsequent color development with TMB substrate, the absorbance (OD value) is measured at 450nm/630nm wavelengths using an ELISA reader. The percent absorbance is calculated, and the concentration of kanamycin in the sample is inversely correlated with the percent absorbance.

Performance specifications

Detection Limit: < 0.05 ng/mL

Quantification Limit: 0.05 ng/mL

Linear Range: 0.05 - 5 ng/mL

Accuracy (Recovery Rate): 70% - 130%

Accuracy (Measurement Deviation): ≤ 15%

Reproducibility (Within-Batch Variation): ≤ 15%

Materials provided

Kit components	Size (96 Tests)	Storage conditions
Microplate	8 × 12	2-8 °C protected from light
Standard solution (50 ng/mL)	1 mL × 1	2-8 °C protected from light
Antibody working solution	7 mL × 1	2-8 °C
Enzyme-labeled secondary antibody	12 mL × 1	2-8 °C
Sample diluent	30 mL × 1	2-8 °C
Wash Buffer (20x)	30 mL × 1	2-8 °C
Developing solution A	8 mL × 1	2-8 °C protected from light
Developing solution B	8 mL × 1	2-8 °C protected from light
Stop solution	15 mL × 1	2-8 °C
Microplate Sealers	3	

Note: The kit should be stored light-protected at 2-8°C. After opening, any unused reagents should still be stored light-protected at 2-8°C. Please refer to the kit label for the production date and expiration date.

Procedure

Reagents / Equipments required but not supplied

Allow the kit components to equilibrate at room temperature for 30 minutes before starting the procedure.

1. Required Consumables and Equipment: Microplate reader, shaking incubator (or incubator), plate washer (hand washing is acceptable), vortex mixer, timer.
2. High-precision pipettes and disposable tips (0.5-10 µL, 10-100 µL, 30-300 µL, 100-1000 µL).
3. Deionized water.

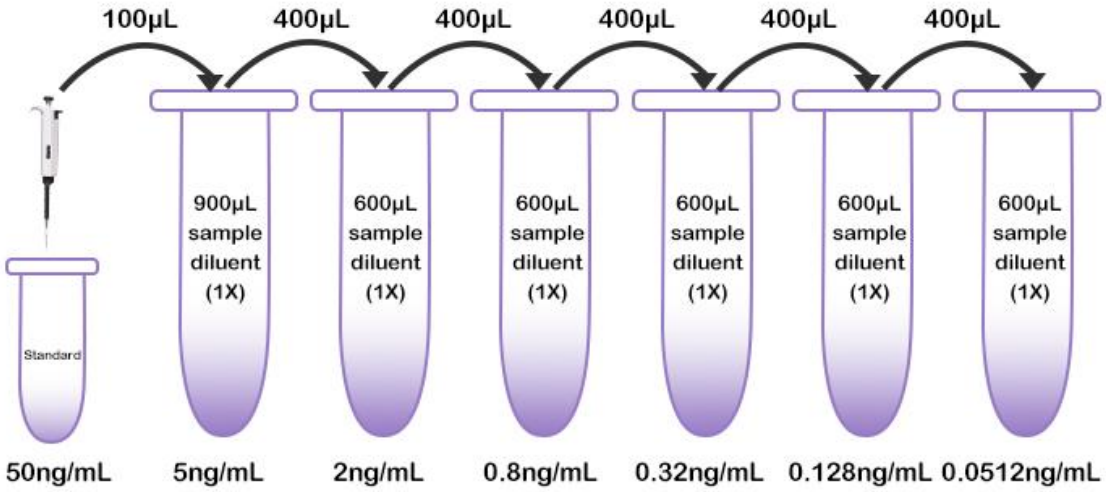
4. Absorbent paper, EP tubes, disposable gloves.

Reagent preparation

1. Preparation of Wash Buffer (1×): Take 1 part of the 20× Wash Buffer and dilute it with 19 parts of deionized water to prepare a working concentration of Wash Buffer (1×). If crystals form in the 20× Wash Buffer, gently shake it at room temperature or in a 37 °C water bath until the crystals are completely dissolved before dilution. Unused 20× Wash Buffer should be stored at 2-8 °C.

2. Preparation of Color Development Solution: Mix the same volumes of Development Solution A and Development Solution B, and mix thoroughly. Place the mixture in a light-protected area. **(Note: Do not leave the mixture for too long; it is generally prepared within 10 minutes before use. If the mixed color development solution turns blue, do not use it).**

3. Preparation of Standard Solution: Dilute the standard solution to 5 ng/mL using the Sample Diluent, and then prepare the standard solution by diluting it 2.5-fold (prepare a fresh standard solution for each experiment). Refer to the diagram below:



Sample preparation

Prepare the sample at room temperature and mix it well before adding. If the user needs to dilute the sample or the high-concentration standard provided in the kit, dilute it using the Sample Diluent provided in the kit. For cell-based samples, it is recommended to centrifuge at 3000 rpm for 5 minutes and use the supernatant for testing.

Assay Procedure

All operations should be conducted at room temperature, and it is recommended to perform duplicate measurements for all sample wells.

1. Allow the reagents in the kit to equilibrate at room temperature for 30 minutes. Take out the required microplate strips from the aluminum foil bag that has been equilibrated to room temperature. Use a marker pen to label the sequence of the plate strips (it is recommended to perform duplicate well measurements). After sealing the remaining plate strips with a plate sealer, place them back into the aluminum foil bag, seal it, and store at 2-8 °C.

Note: The plate strips are prone to detachment during subsequent tapping steps, so be sure to label them properly.

2. Sample incubation: Add 50 µL of standard / blank control (sample diluent) / samples to each well, followed by the addition of 50 µL of antibody working solution. Seal the plate with a plate sealer and incubate it at 25 °C in a light-protected area for 30 minutes.

Note: It is necessary to add the standard first; if the antibody is added first, it will directly react with the antigen on the plate. Incomplete sealing or lack of sealing during incubation can lead to evaporation of the reaction solution and result in experimental errors. Avoid exposing the plate to light during the incubation process.

3. Plate washing: After incubation, carefully remove the plate sealer and discard the liquid from the wells. Wash the plate three times with Wash Buffer (1×) (250µL/well) and tap dry to remove residual liquid from the wells.

Note: If hand washing is used, add the Wash Buffer (1×) while suspending the plate, and try to avoid touching the inner walls of the wells with the pipette tip. After each addition of Wash Buffer (1×), let it stand for 30 seconds and gently shake. When tapping dry, use fresh absorbent paper each time or tap dry in a clean area of the paper.

4. Enzyme-labeled secondary antibody incubation: Add 100 µL of enzyme-labeled secondary antibody to each well, seal the plate with a plate sealer, and incubate it at 25 °C in a light-protected area for 30 minutes.

5. Plate washing: Same method as step 3.

6. Color development: Add 100µL of pre-mixed color development solution to each well, seal the plate with a plate

sealer, and incubate it at 25 °C in a light-protected area for 15 minutes.

7. Stop reaction: Add 100 µL of stop solution to each well. After uniform color development, the plate is ready for reading.

Note: It is recommended to set a shaking step of 5-10 seconds in the reading program of the plate reader.

8. Reading: Place the microplate in the plate reader, set the dual-wavelength at 450/630 nm, and read the absorbance values. The measurement should be completed within 20 minutes after termination.

Calculation of results

1. Calculation of Absorbance Values

The absorbance value for each standard or sample is calculated as $OD_{450\ nm}$ - $OD_{630\ nm}$.

2. Calculation of Percentage Absorbance

The average absorbance value (dual wells) of each standard or sample is divided by the average absorbance value of the 0ng/mL standard, and then multiplied by 100 % to obtain the percentage absorbance:

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

B: average absorbance value of the standards or samples

B_0: average absorbance value of the 0 ng/mL standard

3. Construction and Calculation of the Standard Curve

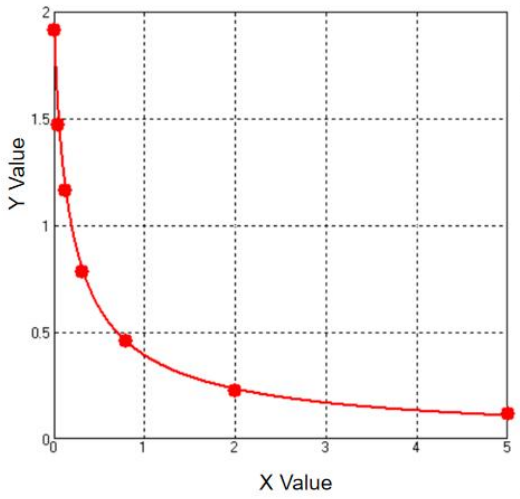
Plot the percentage absorbance values of the standards on the Y-axis and the concentration values of the standards on the X-axis to construct the standard curve. It is recommended to use a four-parameter logistic mathematical model for curve fitting with the equation:

$$Y = (A - D) / (1 + (X / C) ^ B) + D$$

Substitute the percentage absorbance value of the sample into the standard curve to determine the corresponding concentration value of the sample. Multiply it by the corresponding dilution factor to obtain the actual concentration of kanamycin in the sample.

Example Display

The following standard curve graph is for reference only. The actual standard curve should be based on the standard curve plotted using the standards from the same experiment.



Standard curve (ng/mL)	Percentage absorbance (%)
5	5.90
2	11.66
0.8	22.42
0.32	40.05
0.128	59.63
0.0512	76.89
0	100

Precautions

1. The optimal reaction temperature for this kit is 25 °C. Deviations from this temperature, either higher or lower, may result in changes in absorbance values and sensitivity.
2. All components in the kit must be equilibrated to room temperature (20-25 °C) before use.
3. Thoroughly mix all components before use, and briefly centrifuge the standard substance for 5 seconds to collect any liquid on the tube walls and cap at the bottom. Immediately return all reagents to 2-8 °C after use.
4. The kit must be used within its expiration date, and a corresponding standard curve should be prepared for each assay. It is not recommended to mix reagents from different batches.
5. When adding liquids to the microplate, avoid touching the bottom of the wells to prevent damage to the coating. Promptly replace the sample wells and tips between different samples and steps to avoid cross-contamination.
6. Take care to prevent strip detachment when drying the plate after washing, and do not reuse the plate sealer.
7. During color development, the presence of black flocculent material may occur at high concentrations, which is a normal phenomenon. Mild occurrences do not affect the final reading results.
8. During reading, ensure that the detection wavelength and fitting equation are correctly selected.
9. Strict adherence to the instructions and the use of all reagents provided in the kit are necessary to ensure optimal

detection results.

10. Different factors, including operator technique, pipetting methods, plate washing techniques, reaction time or temperature, and kit storage, can contribute to variations in the test results.

11. Our company is only responsible for the kit itself and not for sample consumption caused by using the kit. Users should consider the potential sample usage and reserve an adequate amount of samples before use.

12. This kit is for in vitro research use only and is not intended for clinical diagnosis.

Safety Tips

1. The termination solution in the kit is an acidic solution. Extra caution should be taken during handling.

2. All biological samples carry potential biohazard risks. Users should strictly follow local laws and regulations for handling and disposal of samples.

3. For safety purposes, operators should wear personal protective equipment, such as lab coats, gloves, masks, and goggles.

Common Problems and Analysis

If there are issues with the experimental results, promptly take photos of the color development results and properly store unused strips and reagents. Then, contact technical support for assistance. You can also refer to the following information to identify the possible causes.

Problem description	Possible reasons	Corresponding countermeasures
Gradient bad in the standard curve	Incorrect dilution	Failure to dilute the standard curve according to the specified dilution factor
	Inaccurate liquid aspiration or addition	Check the pipette and tips
	Incomplete enzymatic plate washing	Ensure the appropriate frequency of plate washing and the volume of wash buffer per well
Weak or colorless color development	Insufficient incubation time	Ensure an adequate incubation time
	Incorrect experimental temperature	Use the recommended incubation temperature
	Insufficient or missed addition of reagents	Check the liquid aspiration and addition process to ensure all reagents are added in the correct order and in sufficient amounts
	Incorrect preparation of the color development solution	Mix equal volumes of color development solution A and B without exposure to light, prepare 10 minutes before color development
Low OD (Optical	Incorrect settings on the plate reader	Check the wavelength and filter device on the plate reader

Density) reading		Preheat the plate reader in advance before taking readings
High coefficient of variation (CV) value	Incorrect liquid addition	Check the liquid addition process
	Contamination on the bottom of the microplate	Check for residual liquid and fingerprints on the bottom of the microplate
	Foreign particles or bubbles in the wells	Ensure there are no foreign particles in the wells before sample addition and confirm the absence of bubbles after sample addition
	Unsealed or incomplete sealing of the microplate during incubation	Seal the microplate with a sealing film
High background value	Incomplete enzymatic plate washing	Follow the recommended washing method in the instructions.
		If using an automated plate washer, check for blockages in all liquid addition and waste disposal ports
		If washing manually, increase the number of washes appropriately
		Insufficient washing or incomplete washing can lead to high background
	Incubation time and temperature	Strictly follow the instructions for the recommended incubation conditions
	Contamination of consumables	Ensure the cleanliness of the tubes, tips, and other consumables used
	Contamination in the washing solution	Prepare fresh washing solution
	Contamination of the color development solution	The color development solution itself should be colorless. Ensure that the color development solution is not contaminated with metal ions or oxidizing reagents before use and store it in a light-protected manner
Low sensitivity	Improper storage of the kit	Store the relevant reagents according to the instructions

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.