EZElisa™ Gentamicin Residual ELISA Kit

Cat #: A-QEK008

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

Gentamicin is an aminoglycoside antibiotic used in the treatment of animal diseases, strain selection, and preparation of cell and gene therapy drug raw materials. However, it has neurotoxic, nephrotoxic, and ototoxic effects, leading to vestibular and cochlear damage. Residues of gentamicin in animal products and biopharmaceuticals can affect human health and even cause allergic reactions. Both European and American countries, as well as China, have restrictions on its use. This kit is designed for the detection of gentamicin residues in cell and gene therapy drug raw materials, intermediates, semi-finished products, and finished products. For the testing of other biopharmaceutical samples, it is recommended that users perform suitability verification to eliminate interference from matrices.

Principles

This assay kit utilizes an indirect competitive ELISA method to detect trace amounts of gentamicin residues in the sample. The microplate is pre-coated with a conjugated antigen, and the residual gentamicin in the sample competes with the conjugated antigen on the microplate for anti-gentamicin antibodies. After the addition of enzyme-labeled secondary antibody, the color is developed by adding TMB substrate, and the absorbance (OD value) is measured using a plate reader at 450 nm/630 nm wavelengths. The absorbance is inversely correlated with the concentration of gentamicin in the sample.

Performance specifications

Detection Limit: < 0.1 ng/mL

Quantification Limit: 0.1 ng/mL

Linear Range: 0.1-10 ng/mL

Accuracy (Recovery Rate): 70% - 130%

Accuracy (Measurement Deviation): ≤ 15%

Reproducibility (Within-Batch Variation): ≤ 10%

Materials provided

Kit components	Size (96 Tests)	Storage conditions
Microplate	8 × 12	2-8 °C protected from light
Standard solution (100 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.

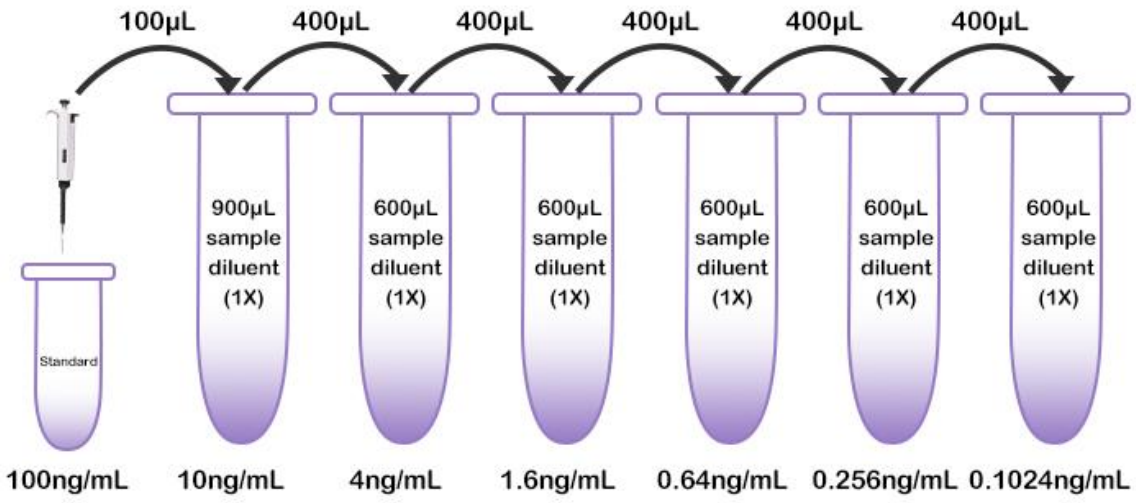
4. Absorbent paper, EP tubes, disposable gloves.

Reagent preparation

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

2. Preparation of Developing Solution: Mix the same volumes of Developing solution A and Developing solution B, and mix well. 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 Developing Solution has turned blue, do not use it.)**

3. Preparation of Standard Solutions: Dilute the standard stock solution to 10ng/mL using the sample diluent. Then, prepare the standard solutions by further diluting them with a 2.5-fold dilution factor (prepare a fresh standard solution for each experiment). Refer to the diagram below:



Sample preparation

Recover the samples to room temperature and mix them well before adding to the assay. If the user needs to dilute the samples or the high-concentration standard provided in the kit, they can use the sample diluent provided in the kit for dilution. For cell-based samples, it is recommended to centrifuge at 3000rpm for 5 minutes, collect the supernatant,

and use it for testing.

Assay Procedure

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

Note: The strips are prone to detachment during subsequent plate tapping, so pay attention to proper labeling.

2. Sample incubation: Add 50 µL of standards / 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 sealing film and incubate at 37 °C in a light-protected area for 30 minutes.

Note: It is necessary to add the standards first. Adding the antibody first will result in direct reaction with the antigen on the plate. During incubation, incomplete sealing or lack of sealing will lead to evaporation of the reaction solution and experimental errors. Avoid exposure to light during all incubation steps.

3. Plate washing: After incubation, carefully remove the plate sealing film and discard the liquid in the wells. Wash the plate 3 times with wash buffer (1×) (250 µL/well) and tap to remove residual liquid in the sample wells.

Note: If washing the plate manually, add the wash buffer (1×) without touching the well walls. After adding the wash buffer (1×), let it sit for 30 seconds and gently shake. During tapping, use fresh absorbent paper each time or tap 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 at 37 °C for 30 minutes.

5. Plate washing: Same method as step 3.

6. Color development: Add 100 µL of the pre-prepared chromogenic substrate to each well, seal the plate with a plate sealer, and incubate at 37 °C in a light-protected area for 15 minutes.

7. Stop reaction: Add 100 µL of stop solution to each well, mix well, and read the absorbance.

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

8. Reading: Place the microplate in the ELISA reader, set the wavelength to dual wavelength 450/630 nm, and read the absorbance values. The measurement should be completed within 20 minutes after the stop solution is added.

Calculation of results

1. Calculation of Absorbance Values:

The absorbance value for each standard or sample is calculated as OD450 nm - OD630 nm.

2. Calculation of Percentage Absorbance

The average absorbance value (dual wells) for each standard or sample is divided by the average absorbance value of the 0 ng/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 0ng/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 fitting the equation:

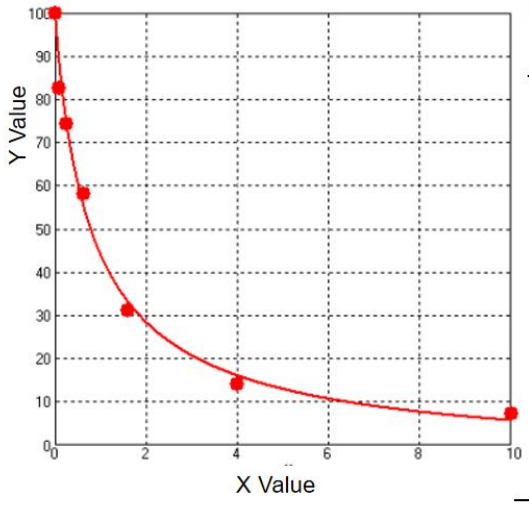
$$Y = (A - D) / (1 + (X / C) ^ B) + D$$

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 the gentamicin in the sample.

Note: Whenever possible, use the recommended dual-wavelength correction method, with correction performed using a wavelength of 630nm. OD_{450nm} - OD_{630nm} is the corrected OD value, which can be directly used for calculations or further blank correction based on data quality. If a dual-wavelength ELISA reader is not available, after reading the OD450 data, the data quality should be assessed before performing blank correction.).

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 (%)
10	7.38
4	14.19
1.6	31.01
0.64	58.04
0.256	74.26
0.1024	82.67
0	100.00

Precautions

1. All components of the kit must be equilibrated to room temperature (20-25 °C) before use.

2. Thoroughly mix all components before use. Briefly centrifuge the standard for 5 seconds to collect any liquid on the tube walls and cap. Immediately return all reagents to 2-8 °C after use.

3. The kit should be used within its expiration date. A corresponding standard curve should be prepared for each assay, and it is not recommended to mix reagents from different batches.

4. When adding solutions to the microplate, avoid touching the bottom of the wells to prevent damage to the coating. Replace the sample wells and tips between different samples and steps to avoid cross-contamination.

5. When tapping dry the plate strips after washing, be careful to prevent detachment of the strips. The sealing film should not be reused.

6. During color development, the high concentration may produce black precipitates, which is a normal phenomenon. Mild precipitation does not affect the final reading results.

7. When reading the results, ensure that the detection wavelength and the selected fitting equation are correct.

8. Strictly follow the instructions and use only the reagents provided in the kit to ensure optimal detection

performance.

9. Different results can be caused by various factors, including operator technique, pipetting method, plate washing technique, reaction time or temperature, and reagent storage.

10. Our company is only responsible for the kit itself and is not responsible for sample consumption caused by using the kit. Users should consider the potential sample volume and reserve sufficient samples before use.

11. 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 Density) reading	Incorrect settings on the plate reader	Check the wavelength and filter device on the plate reader
		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.