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Instructions for Use of Gentamicin Residual ELISA Detection Kit

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG-GE001

Introduction

This kit determines the trace residue of Gentamicin in samples by indirect competitive ELISA. The plate is pre-coated with conjugated antigen. Gentamicin remaining in the sample and conjugated antigen pre-coated on the plate strips compete for anti-Gentamicin antibody. Add enzyme-labeled secondary antibody, and then add TMB substrate for color development. Measure the absorbance (OD value) at 450 nm/630 nm using a plate reader, and calculate the percent absorbance. The concentration of Gentamicin in the sample is negatively correlated with the percent absorbance.

Assay range : 0.1 - 10 ng/mL
Limit of quantitation : 0.1 ng/mL
Limit of detection : < 0.1 ng/mL
Accuracy : 70% - 130%

Specification

96T

Usage

This kit is suitable for quantitative detection of residual Gentamicin content in drug substance, intermediates, and drug products of cell and gene therapy drugs.

Kit components

Components	Specification	Preparation
Gentamicin Standard (100 ng/mL)	1 mL × 1 tube	Gradient dilution with sample diluent
Coated Plate	8 wells × 12 strips	Ready-to-use
Sample Diluent	30 mL × 1 vial	Ready-to-use
Wash Buffer (20 x)	50 mL × 1 vial	Make a 20-fold dilution with ultrapure
Detection Antibody	7 mL × 1 vial	Ready-to-use
Streptavidin-HRP	12 mL × 1 vial	Ready-to-use
TMB Substrate A	8 mL × 1 vial	Ready-to-use
TMB Substrate B	8 mL × 1 vial	Ready-to-use
Stop Solution	10 mL × 1 vial	Ready-to-use
Sealing Film	5 pieces	Ready-to-use
Instructions for Use	1 copy	Ready-to-use

Storage and shelf life

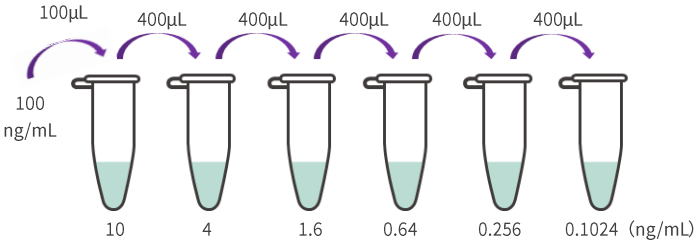
The kit should be stored at 2-8℃ away from light. The shelf life is 12 months. After opening, unused kits should be stored at 2-8℃ away from light.

Apparatus and materials to be prepared by the user:

- Plate reader
- Thermostat plate shaker
- Micro pipette and tips
- Deionized water
- Unused filter paper
- Vortex shaker

Reagent preparation

1. All reagents and samples to be tested should be restored to room temperature. All reagents are newly prepared and ready-to-use.
2. Preparation of 1x washing solution : Equilibrate the concentrated washing solution to room temperature, without crystallization. After mixing well, according to the usage volume, dilute 20x washing solution by 20 times with an appropriate amount of ultrapure water at the ratio of 1:19, to obtain 1x washing solution.
3. Preparation of color reagent : Mix equal volumes of color reagent A and color reagent B, mix well and place the mixture in the dark. (Note: It cannot be left for too long and is generally prepared 10 min before use. Do not use if the mixed color reagent has turned blue).
4. Preparation of standard : Dilute the standard with sample diluent buffer to 10 ng/mL, and then prepare the standards by 2.5-fold dilution (freshly prepared standard solution shall be used for each experiment), as shown in the figure below:



Operating procedures

All reagent components and samples to be tested should be restored to room temperature before use. Duplicate well assay is recommended for all standards and samples to be tested.
1. Preparation of reagents: Prepare all reagents to be tested, diluted standards and samples to be tested in advance.
2. Microplate strip determination: Calculate the microtiter strips required for the samples to be tested and standards, remove the microtiter strips from the aluminum foil bag, place the remaining microtiter strips back into the aluminum foil bag and seal the mouth of the bag, and store it at low temperature.
3. Sample incubation: Add 50 μL of standard, blank (sample diluent buffer) and sample to each well, add 50 μL of detection antibody to each well, seal the plate with plate sealer, and then place it at 37℃ away from light for 30 min
4. Plate washing: Discard the liquid in the wells, add 1 × washing solution (250 μL/well) to wash the plate for 3 times, and pat dry the residual liquid in the microtiter plate.
5. Enzyme conjugate incubation: Add enzyme conjugate into microtiter plate with 100 μL/well, seal the plate with sealing film, incubate for 30 minutes at 37℃.
6. Plate washing: Same as Step 4.
7. Color development: Add the prepared color reagent into the plate according to 100 μL/well, seal the plate with plate sealer, and incubate at 37℃ away from light for 15 min.
8. Termination: Add 100 μL stop solution into each well, and gently shake the microtiter plate until the color development is uniform.
9. Readings: Read the absorbance value at 450 nm/630 nm within 20 minutes. Take 450 nm as detection wavelength and 630

nm as reference wavelength..

Result processing

1. Calculation of absorbance value

The calculation formula of absorbance value of each standard or sample: OD450nm - OD630nm.

2. Calculation of percent absorbance

Divide the average absorbance value of each standard or sample (duplicate wells) by the average absorbance value of the 0 ng/mL standard and multiply by 100% to obtain the percent absorbance:

$$\text{Percent absorbance (\%)} = B/B0 \times 100\%$$

B: Average absorbance value of standard or sample
B0: Mean absorbance value of 0 ng/mL standard

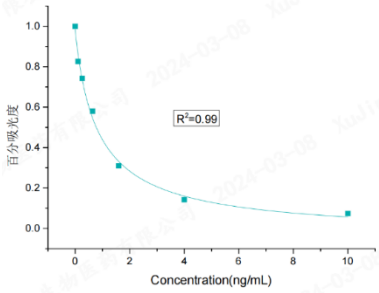
3. Drawing and calculation of standard curve

Draw the standard curve by taking the percent absorbance value of the standard as Y-axis and the concentration value of the standard as X-axis. It is recommended to use the four-parameter logistic mathematical model to fit the equation:

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

Substitute the percent absorbance value of the sample into the standard curve, read the concentration value corresponding to the sample, and multiply its corresponding dilution factor to obtain the actual concentration of Gentamicin in the sample

Standard Curve (ng/mL)	Percent 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



(The above standard curve is only for reference, and the standard curve drawn from the standard of the same experiment should prevail)

Precautions

1. When the sample is tested for the first time, it is recommended to perform dilution with at least 3 consecutive dilution factors, so as to generate at least one diluted sample within the range of the standard curve.
2. The reagents should be stored as indicated on the label, and should be equilibrated to room temperature before use.
3. Before using the coated microtiter plates, please equilibrate to room temperature and then open the secondary packaging. The strip plates not used in the test should be immediately placed back into the package and sealed properly, and can be stored at 4℃ for one month. Other unused reagents should be packaged or covered properly.
4. Please use disposable tips during experimental operation to avoid cross contamination.
5. Please check each individual reagent in the kit fully before use. To obtain accurate assay results, it is of special importance to mix well or shake well the reagents for dilution, loading, and reaction termination.
6. When washing residual Wash Buffer in the reaction wells, pat the plate dry adequately on clean tissue papers until watermark is no longer visible. Do not put the tissue paper into the well for liquid absorption.
7. The TMB Substrate is photosensitive, thus long-time exposure to illumination should be avoided; avoid contact with metal, otherwise, the assay results may be affected.
8. The kit is intended for single use. Please use within the shelf life.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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