Instructions for Use of Kanamycin ELISA Detection Kit

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

Cat. No. HG-KA001

Introduction

BlueKit® series Kanamycin ELISA Detection Kit is a specialized kit for quantitative detection of residual kanamycin content in drug substance, intermediates, and drug products of cell and gene therapy drugs.

This kit determines the trace residue of kanamycin in samples by indirect competitive ELISA. The plate is pre-coated with conjugated antigen. Kanamycin remaining in the sample and conjugated antigen pre-coated on the plate strips compete for anti-kanamycin 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 kanamycin in the sample is negatively correlated with the percent absorbance.

Assay range: 0.05 - 5 ng/mL

Limit of quantitation: 0.05 ng/mL

Limit of detection: < 0.05 ng/mL

Precision: CV% ≤ 10%, RE% ≤ ±15%

Specification

96T

Usage

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

Kit components

Components	Specification	Preparation
Coated Plate	8 wells × 12 strips	Ready-to-use
Kanamycin Standard (Kan Standard) 50 ng/mL	1 mL × 1 tube	Ready-to-use
Detection Antibody (Anti-Kan Biotin)	7 mL × 1 vial	Ready-to-use
Enzyme-labeled secondary antibody (Streptavidin HRP)	12 mL × 1 vial	Ready-to-use
Sample Diluent Buffer	30 mL × 1 vial	Ready-to-use
20× Wash Buffer	30 mL × 1 vial	1:19, dilute with deionized water
Color Reagent A	8 mL × 1 vial	Ready-to-use
Color Reagent B	8 mL × 1 vial	Ready-to-use
Stop Solution	15 mL × 1 vial	Ready-to-use
Plate Sealer	5 pieces	
Instructions for Use	1 copy	

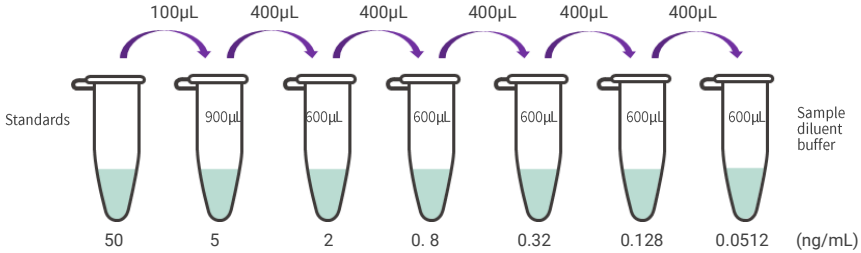
Notes: The kit should be stored at 2-8℃ away from light. The shelf life is 12 months. After opening, unused kits should be stored at 2-8℃ away from light.

Apparatus and materials to be prepared by the user:

(1) Plate reader
(2) Thermostat incubator
(3) Micro pipettes and tips
(4) Deionized water
(5) New filter paper
(6) Vortex oscillator

Reagent preparation

(1) Preparation of wash buffer (1×)
Measure an appropriate amount of 20× wash buffer and dilute with deionized water at 1:20. If crystals are formed in the wash buffer (20×), gently shake it at room temperature or 37℃ water bath, and dilute it after crystals are completely dissolved.
(2) 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).
(3) Preparation of standard curve:
Dilute the standard with 100 μL standard + 900 μL sample diluent buffer to 5 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

(1) Restore each component of the kit to room temperature for 30 min, take out the plate strips required for the test from the aluminum foil bag that has been equilibrated to room temperature, mark the order of the plate strips with a marker (it is recommended to perform replicate determination), seal the remaining plate strips with a plate sealer and then put it back into the aluminum foil bag, seal and store at 2-8℃. (Note: In the subsequent step of plate patting, the plate strips are easy to fall off, so be sure to mark them well)
(2) 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 25℃ away from light for 30 min. (Note: Standards must be added first, as antibodies will directly react with antigens on the plate if they are added first; if the plate is not sealed or the plate is not completely sealed during the incubation, it will lead to evaporation of the reaction buffer, resulting in experimental errors; light exposure shall be avoided as much as possible during the whole incubation period)
(3) Plate washing: After incubation, carefully remove the plate sealer, discard the liquid in the wells, wash the plate 3 times with washing buffer (1×) (250 μL/well), and pat dry the residual liquid in the sample wells. (Note: If the plate is washed by hands, it is necessary to hang the tip in the air and avoid it touching the inner wall of the well when adding the wash buffer (1×); allow to stand for 30 s and slightly shake after adding the wash buffer (1×) each time; pay attention to changing a new absorbent paper each time or patting on the clean area on the paper when patting dry)

(4) Incubation of enzyme-labeled secondary antibody: Add enzyme-labeled secondary antibody to each well, 100 μL/well, seal the plate with plate sealer, and then incubate at 25°C away from light for 30 min.

(5) Plate washing: Method is the same as Step (3)

(6) Color development: Add the prepared color reagent into the plate according to 100 μL/well, seal the plate with plate sealer, and incubate at 25℃ away from light for 15 min.

(7) Termination: Add stop solution, 100 μL/well, and read after color of solution is uniform. (Note: It is recommended to set 5 - 10 s oscillation in reading procedure of plate reader)

(8) Reading: Place the plate in the reader, set the wavelength as dual wavelength 450/630 nm, and read the absorbance value. The determination shall be completed within 20 min after termination.

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

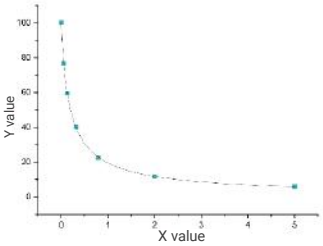
Draw the standard curve by taking the percent absorbance value of the standard as Y-axis and the concentration value of the standard as X-axis. It is recommended to use the four-parameter logistic mathematical model to fit the equation:

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

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 kanamycin in the sample

✧ Example:

Standard Curve (ng/mL)	Percent Absorbance (%)
5	5.9
2	11.66
0.8	22.42
0.32	40.05
0.128	59.63
0.0512	76.89
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) The optimal reaction temperature of the kit is 25°C. Too high or too low temperature will lead to changes in the detected absorbance and detection sensitivity.

(2) Allow all components in the kit to return to room temperature (20-25℃) before use.

(3) Mix all components well before use, and centrifuge the standard briefly for 5 s to allow all the liquid on the wall of the tube and the cap to concentrate at the bottom of the tube; put all reagents at 2-8°C condition immediately after use.

(4) The kit must be used within the shelf life, and the corresponding standard curve needs to be prepared for each test. It is not recommended to mix different batches of relevant reagents for batch use.

(5) When loading solution into the plate, pay attention not to touching the bottom of the plate to prevent damage to the coating layer. Change the reagent reservoir and tips between loading of different samples and between steps in time to avoid cross-contamination.

(6) When patting the plate strip after washing, pay attention to preventing the strip from falling off, and the plate sealer should not be reused.

(7) Black floccules may be produced at high concentrations during color development, which is normal, and few floccules will not affect the final reading result.

(8) Check whether the detection wavelength and fitting equation are selected correctly when reading.

(9) The best detection effect can be ensured only by strictly following the instructions and using all the reagents provided with this kit.

(10) The difference in detection results can be caused by a variety of factors, including the operation of laboratory personnel, the use mode of pipettes, plate washing technique, reaction time or temperature, and the storage of kits.

(11) Our company is only responsible for the kit itself and not for the sample consumption caused by the use of the kit. Users should fully consider the possible use of the sample before use and reserve sufficient samples.

(12) This kit is for in vitro research use only and is not used for clinical diagnosis.

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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