Kanamycin ELISA Kit - Instruction

Catalog Number : ARK6617

【Intended use】

This kit is suitable for the detection of kanamycin residues in raw materials, intermediates, semi-finished products and finished products of cell and gene therapy drugs. For the testing of other biological product samples, users are advised to perform suitability verification to exclude matrix interference.

【Principle】

This kit uses indirect competitive ELISA method to determine the trace residue of kanamycin in samples. The conjugate antigen was pre-coated on the microbar, and the residual kanamycin in the sample and the conjugate antigen pre-coated on the microbar competed with the anti-kanamycin antibody, adding the enzymo-labeled secondary antibody, and then the absorbance (OD) was determined at the wavelength of 450nm/630nm by adding TMB substrate. Absorbance was negatively correlated with kanamycin content in the sample.

【Materials provided】

Number	Component	Size	Storage condition
1	Microtiter Plate	96 T	2-8℃ (keep in dark)
2	Standard (50 ng/mL)	1 mL×1 tube	2-8℃ (keep in dark)
3	Sample Dilution	30 mL×1 vial	2-8℃
4	Enzyme-labeled Secondary Antibody	12 mL×1 vial	2-8℃
5	Antibody Working Solution	7 mL×1 vial	2-8℃
6	Wash Buffer (20×)	30 mL×1 vial	2-8℃
7	Developing Solution A	8 mL×1 vial	2-8℃ (keep in dark)

8	Developing Solution B	8 mL×1 vial	2-8℃ (keep in dark)
9	Stop Solution	15 mL×1 vial	2-8℃
10	Microplate Sealers	3	-

【Storage】

The kit should be stored away from light at 2-8 ° C for 12 months. The unused kits should be kept away from light at 2-8 ° C after opening.

The date of manufacture and expiration date are shown on the kit label.

【Reagents/equipments required but not supplied】

1. Enzyme-labeled instrument, thermostatic oscillator (or thermostatic incubator), plate washer (or hand washing), vortex oscillator, timer
2. Pipettes and pipette tips (0.5- 10 μL, 10- 100 μL, 30-300 μL, 100- 1000 μL)
3. Deionized or distilled water.
4. Bibulous paper. EP tubes, disposable gloves.

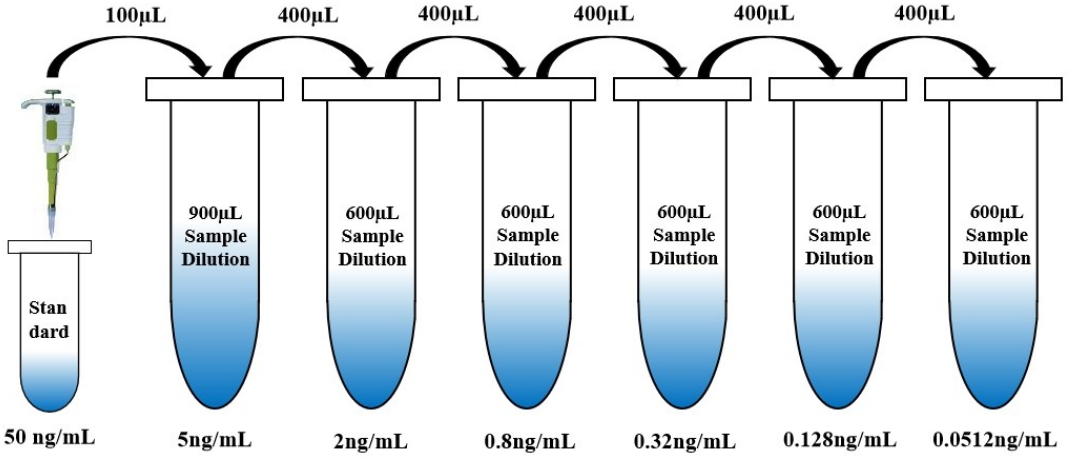
【Reagent preparation】

- Wash buffer (1×): add the deionized water into the wash buffer (20×) to dilute for 20 fold for standby, for example, add 190 mL of deionized water into 10 mL wash buffer (20×), and mix well.If there are crystals formed in the wash buffer (20×), it shall be placed at room temperature or 37℃ water bath and shaken gently to allow the crystals to dissolve completely before dilution.
- Substrate Solution: Mix the developing solution A and developing solution B with equal volume before 10 minutes using. Avoid light to ensure that the substrate solution is not contaminated. If the substrate solution after mixing has turned blue, do not use it.
- Standard: The standard (50 ng/ml) is diluted to 5 ng/ml with the sample diluent, and then the subsequent standards are prepared by serial ratio (dilution: 2.5-fold) as shown below, duplicate well detection.
- Restore the sample to room temperature, mix the sample before adding; If the user needs to dilute the sample or the high concentration standard in the box, the sample diluent in the box can be used for dilution; For cell samples, it is recommended to centrifuge at 3000rpm for 5min before testing and take the supernatant for testing.

【Assay procedure】

Bring all reagents and samples to room temperature before use.

1. Bring the test slats from the aluminum foil bag that has been balanced to room temperature, put the remaining slats back into the aluminum foil bag, seal them, and store them in 2-8℃ .
2. Preparation of the standard curve: dilute the Standard with Sample dilution to 5 ng/mL, then prepared by Multiple Dilution method.



3. Sample incubation: Add 50μL standard/blank (sample diluent)/sample to each well, then add 50μL antibody working solution, seal the plate with a sealing plate film, and then place it at 25℃ for 30min away from light.(Note: the standard product must be added first, if the antibody is added first, it will react directly with the antigen on the plate).
4. Washing board: discard the liquid in the well and wash the board with wash buffer (1×) for 3 times (250 μL /well). Pat dry residual liquid in sample well. (Before discarding the liquid in the well each time, if washing plate by hand, the liquid can be left for 1min after adding and slightly shaken; If plate washing machine is used, 5 s can be slightly shaken after adding wash buffer).
5. Enzyme-labeled secondary antibody incubation: Add enzyme-labeled secondary antibody to each well, 100 μL/ well, seal the plate with sealing plate film, and then incubate at 25℃ for 30 min.
6. Washing board: The method is the same as step 4.
7. Color development: the pre-prepared substrate solution was added to the enzyme label plate, mixed well (100 μL/well), and incubated in darkness ,25℃ for 15 min.
8. Termination: add stop solution (100 μL /well).

9. Recording: The OD value at 450 nm/630 nm should be measured by placing the plate in the microcode, and the reading should be completed within 20 min.

【Calculation of results】

1. Calculation of absorbance value

   The absorbance value of each standard or sample is OD_{450nm}-OD_{630nm}.

2. Calculation of percentage absorbance

   The average absorbance value of each standard or sample (double hole) divided by the average absorbance value of 0ng/mL standard and multiplied by 100%, which is the percentage absorbance:

   Percentage absorbance (%) = B/B0 ×100%

   B: The average absorbance value of a standard or sample

   B0: The average absorbance value of the 0ng/mL standard

3. Drawing and calculation of standard curves (you can draw curves directly with absorbance (OD value) the percentage absorbance))

   Take the percentage absorbance value (or absorbance) of the standard product as the ordinate Y and the concentration value of the standard product as the abscissa X to draw the standard curve. It is recommended to use the four-parameter Logistic mathematical model to fit the equation:

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

   The percentage absorbance value (or absorbance) of the sample is substituted into the standard curve, the corresponding concentration value of the sample is read out, and the actual concentration of kanamycin in the sample is multiplied by its corresponding dilution multiple.

【Precautions】

1. All the components in the kit must be restored to room temperature (20-25℃) before use.
2. Each component shall be mixed well before use, to guarantee uniformness of the reagent, the standards shall be centrifuged for 5 s temporarily, and centralize the liquid on the tube wall and cover onto the bottom of the tube, and place all the reagents back to 2-8℃ immediately after use.
3. The kit must be used within the shelf life, and corresponding standard curve shall be prepared for each test. Mixed use of different batches of related reagents is not recommended.
4. Be careful not to touch the bottom of the microplate during adding liquid to the ELISA plate, to prevent damaging the coating layer. Replace the reagent reservoirs and the tips timely between different samples and steps, to avoid cross contamination.

5. During beating the strips after washing, take care to prevent strips shedding, and the plate sealers shall not be used repeatedly.
6. Black flocs may be generated at high concentration during color development, this is a normal phenomenon, it is minor in degree, it may not affect the final reading results.
7. Note to check whether the correct detection wavelength and fitting equation are selected during reading.
8. The best detection results can only be guaranteed by strictly following the instructions and using all the reagents supplied with the kit.
9. The difference in testing results can be caused by several factors, including operations of the experimenters, using way of the pipettes, plate washing method, reaction time or temperature, storage of the kit etc.
10. Our company is only responsible for the kit itself, but not be responsible for sample consumption caused by using this kit. Please fully consider the possible usage amount of samples before use, and reserve sufficient samples.
11. This kit is only used for in study, not for clinical diagnosis.

【Safety instructions】

1. All the biological samples have potential biosafety risks, the users shall operate, dispose of and discard the samples in strict accordance with the local laws and relevant provisions.
2. For safety reasons, the operators shall wear the personal protective equipment, such as lab coat, gloves, face masks and safety glasses.

【Problem analysis】

If there is any problem with the experimental results, please take photos of the color rendering results in time, and properly preserve the unused slats and reagents. Then contact technical support to find out the reason.

Problem Description	Possible Reasons	Solution
Gradient difference of standard curve	Dilution error	The standard curve is not diluted according to the dilution factor
	The liquids are aspirated or added inaccurately	Check the pipette and tips
	The ELISA plates are not washed	Guarantee the plate washing times, and

	completely	the amount used for washing liquid per well
The color development is weak or colorless	The incubation time is too short	Guarantee sufficient incubation time
	The experiment temperature is not correct	Use the recommended incubation temperature
	The reagent volume is insufficient or miss to add the reagent	Check the liquid aspiration and adding process, and guarantee all the reagents are added sufficiently in sequence
	The developing solution are prepared wrongly	The developing solutions A and B are mixed in same volume and protected from light, which are prepared 10 min before color development
The OD value are of low reading	The plate reader are set incorrectly	Check the wavelength and optical filter device on the plate reader
		The plate reader shall be opened in advance before reading for preheating
The OD value are of high reading	The sample is not diluted	It is recommended to validate the dilution factor of the sample
The coefficient of variation (CV value) is large	The liquid adding is incorrect	Check the liquid adding condition
	There are pollution at the bottom of the ELISA plate	Check the bottom of the ELISA plate for residual liquid or handprint
	There are foreign matters or bubbles in the plate well	Confirm that there are no foreign matters in the plate well before adding samples, and confirm that there are no bubbles after adding samples
	The plate is not sealed or sealed incompletely during incubation	Seal the plate with a plate sealer
The background value is high	The ELISA plates are not washed completely	Wash the plate according to the method recommended in the instruction for use
		If automatic plate washer is used, please check all the liquid adding port and waste liquid outlet for blocking
		If the plate is washed by hands, the plate washing times can be added properly

		Insufficient washing or missing to wash may lead to high background
	Incubation time and temperature	Operate in strict accordance with requirements in the instruction for use
	Consumption pollution	The consumables used (such as tubes, tips etc.) are not clean
	The washing solution is polluted	Prepare fresh washing solution
	The developing solution is polluted	The color developing solution itself does not have colors, please ensure that the color developing solution is not polluted by metal ion or oxidizing agent before use, and it is protected from light.
The sensitivity is low	The kit is stored improperly	Store the related reagents in accordance with the requirements in the instruction for use