RES-A025-EN.01

resDetect™ Gentamicin ELISA Kit

Catalog Number：RES-A025

Pack Size: 96 tests

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

Intended Use

The kit is calibrated against the NIFDC and USP standard.

The kit is developed for the detection and quantitative determination of gentamicin residues in plasmid DNA raw materials and proteins for CGT, vaccine and other biological drugs. It is intended for research use only (RUO).

Background

Gentamicin is extracted from the fermentation culture of the genus Monospora of the actinomycete family, which is a basic compound and is a commonly used aminoglycoside antibiotic, which is widely used in media preparation. It is mainly used clinically to treat bacterial infections. Gentamicin is neurotoxic, nephrotoxic, and ototoxic. Its residues in animal food and biological drugs can affect human health and even cause allergic reactions. European and American countries and China require its limited use.

Principle Of the Assay

The Gentamicin ELISA Kit adopts the competitive ELISA method, and the pre-coated conjugated Gentamicin antigen on the microstrip competes with the residual Gentamicin in the sample to bind the enzyme-labeled anti-Gentamicin monoclonal antibody, and then uses a microplate reader to detect the absorbance value by adding TMB substrate, and the absorbance value is negatively correlated with the content of kanamycin in the sample. The kit only takes about one hour and 20 minutes to operate and has a linear range of 0.25 ng/mL to 8 ng/mL.

Precautions

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. The kit is suitable for kanamycin residue detection in CGT, vaccine and other biological drugs plasmid DNA and protein stocks.
3. For the detection of other biologics samples, user suitability verification is recommended to exclude

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dryness of the matrix interference.

4. Do not use reagents past their expiration date.
5. Do not mix or substitute reagents with those from other kits or other lot number kits.
6. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.
7. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

Materials Provided

Table1. Materials Provided

Catalog	Components	Size (96 tests)	Format	Storage
RES-A025-C01	Gentamicin Coated Plate	1 plate	Solid	2-8℃, avoid light
RES-A025-C02	Gentamicin Standard	64 ng/mL×1.8 mL	Power	2-8℃
RES-A025-C03	HRP-Anti-Gentamicin Antibody	6 mL	Liquid	2-8℃, avoid light
RES-A025-C04	1×Dilution Buffer	50 mL	Liquid	2-8℃
RES-A025-C05	20×Washing Buffer	50 mL	Liquid	2-8℃
RES-A025-C06	Substrate Solution	12 mL	Liquid	2-8℃, avoid light
RES-A025-C07	Stop Solution	7 mL	Liquid	2-8℃

Storage

1. The kit should be stored at 2°C to 8°C upon receiving.
2. The reconstructed Gentamicin standard is stored at -70°C in at least 300 uL per tube and cannot be

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frozen and thawed repeatedly.

3. Find the expiration date on the outside packaging and do not use reagents past their expiration date.

Reagents/Equipment Needed but Not Supplied

Single or multi-channel micropipettes and pipette tips: need to meet 10 μL, 300 μL, 1000 μL injection requirements;

37ºC Incubator;

Single or dual wavelength microplate reader with 450nm and 630nm filter;

Tubes;

Timer;

Reagent bottle;

Deionized or distilled water.

Notice Before Measurement

1. Bring all reagents and samples to room temperature (20℃-25℃) before use.
2. Immediately return all reagents to 4℃ after use.
3. The plates can be opened only after all samples have been prepared, and the unused plates are immediately returned to the sealed bag provided with the kit and stored away from light.
4. According to Table 2, prepare the Gentamicin standard into a storage solution with ultrapure water, dissolve at room temperature for 10 minutes, and shake gently and mix well. The reconstructed kanamycin standard is stored at -70℃ in at least 300 uL per tube and cannot be frozen and thawed repeatedly.

Table 2. Preparation method

ID	Components	Size (96 test)	Storage solution concentration.	Reconstituted water Vol.
RES-A025-C02	Gentamicin Standard	64 ng/mL×1.8 mL	64 ng/mL	1.8 mL

Recommended Sample Preparation

Working Solution Preparation

1. Preparation of 1×Washing Buffer:

Dilute 25 mL 20×Washing Buffer with ultrapure water/deionized water to 500 mL.

2. Sample preparation:

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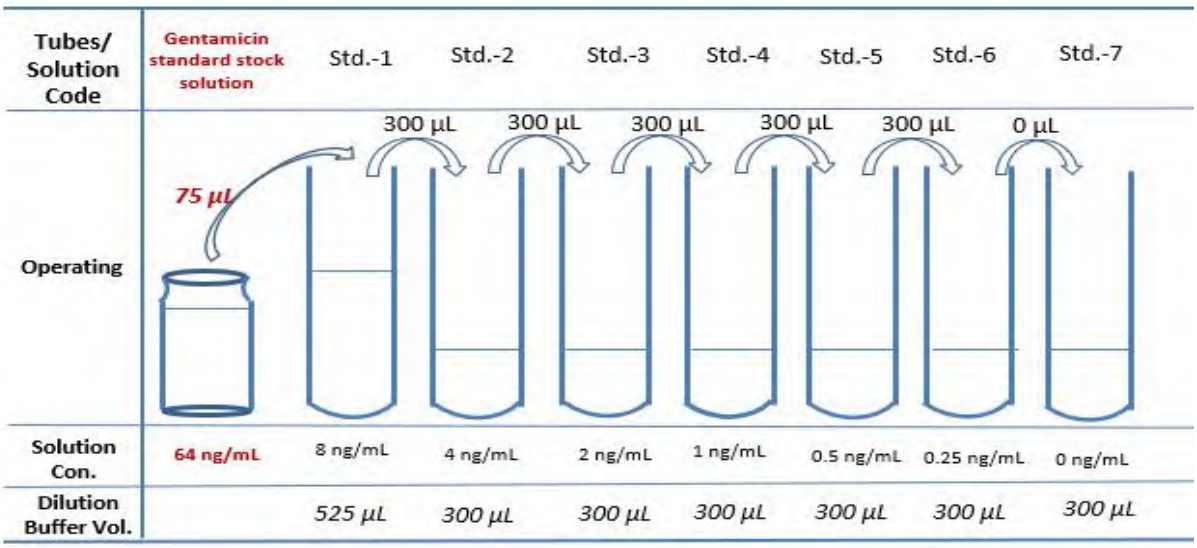
Most samples are diluted according to the dilution ratio confirmed by the interference of the samples themselves.

Procedure for assay

1. Preparation of Standard curve

The Gentamicin standard should be diluted to 8 ng/mL,4 ng/mL,2 ng/mL,1 ng/mL,0.5 ng/mL,0.25 ng/mL,0 ng/mL. Detailed operations are as follows:



2. Add Samples and Antibody

Add 50 µL samples to each well. For Blank Control wells, please add 50 µL Dilution Buffer. Then add 50 µL HRP-Anti-Gentamicin Antibody, seal the plate with microplate sealing film and incubate at 37°C for 1 hour, avoid light.

***Note**: It is recommended to set double holes for samples and standard curves to be tested.*

3. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 2s, remove the supernatant by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

4. Substrate Reaction

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Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at 37℃ for 20 minutes, avoid light.

5. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

Note: *The color in the wells should change from blue to yellow.*

6. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 minutes.

Note*: To reduce the background noise, subtract the value read at* OD_{450nm} *with the value read at* $OD_{630\ nm}$.

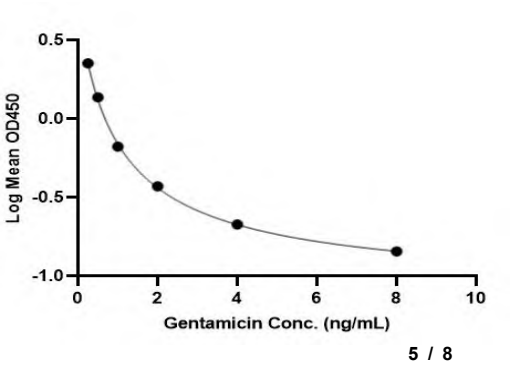
Calculation of Result

1. The standard curve is plotted with the standard concentration as x-axis and Log the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.
2. Normal range of Standard curve: $R^2 \geq 0.9900$.
3. Detection range: 0.25 ng/mL-8 ng/mL. If the OD value of the sample to be tested is higher than 8 ng/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 0.25 ng/mL, the sample should be reported.

Typical Data

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Standard Conc. (ng/mL)	OD450-1	OD450-2	Average
8.00	0.133	0.153	0.143
4.00	0.202	0.222	0.212
2.00	0.354	0.388	0.371
1.00	0.625	0.704	0.665
0.50	1.287	1.443	1.365
0.25	2.223	2.271	2.247



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Linearity and Range

The linear interval was 0.25-8 ng/mL, R^2 > 0.99, and the OD value CV and the CV between the calculated concentration and the theoretical concentration at each concentration point were ≤20%.

Gentamicin (ng/mL)	8	4	2	1	0.5	0.25
OD450	0.133 0.153	0.202 0.222	0.354 0.388	0.625 0.704	1.287 1.443	2.223 2.271
OD450 CV (%)	10	7	6	8	8	2
Relative Error (%)	1	0	-2	4	-4	2

Limit of Quantitation

When the concentration recovery rate was between 80-120% and OD value CV≤20%, the maximum concentration corresponding to 8 ng/mL was confirmed as the upper limit of quantification of the kit (ULOQ). When the concentration recovery rate was between 80-120% and OD value CV≤20%, the corresponding minimum concentration was 0.25 ng/mL, which was confirmed as the lower limit of quantitation (LLOQ) of the kit.

/	Upper limit of quantitation (ULOQ) (8 ng/mL)	Lower limit of quantitation (LLOQ) (0.25 ng/mL)
OD450 CV (%)	3	6
Recovery Rate (%)	102	90

Accuracy

Three samples of different concentration were prepared, and the range of the recovery rate were 80-120%.

Samples	1	2	3
Sample Conc.(ng/mL)	8	2	0.25
Detected Sample Conc. (ng/mL)	8.14	2.32	0.23
Recovery Rate (%)	102	116	90

Precision

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1. Intermediate precision

Two experimenters tested the samples of three different concentrations to evaluate the intermediate precision, and the detection concentration CV were less than 20%.

Experimenter	1			2		
Samples	1	2	3	1	2	3
Sample Conc.(ng/mL)	8	2	0.25	8	2	0.25
Detected Sample Conc. (ng/mL)	8.14	2.32	0.23	10	1.86	0.26
CV (%)	1	11	7	15	5	3

2. Repeatability

Three samples of known concentration were tested ten times to evaluate repeatability, OD value CV≤10% and detection concentration CV≤20%.

Samples	1	2	3
Sample Conc.(ng/mL)	8	2	0.25
OD450	0.145	0.318	1.963
	0.139	0.316	1.987
	0.128	0.295	1.938
	0.131	0.301	1.914
	0.134	0.302	1.949
	0.138	0.309	1.959
	0.137	0.324	2.058
	0.157	0.308	2.111
	0.129	0.291	1.841
	0.124	0.301	2.006
OD450 CV(%)	3	0	2
Detected Sample Conc. (ng/mL)	8.14	1.86	0.23
Concentration CV(%)	1	11	7

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Specificity

1. Cross-reactivity

When 500 μg/mL ampicillin, tetracycline and chloramphenicol were added into the sample diluent, no cross-reactivity was observed.

Cross Reactant	Cross-reactivity
Gentamicin (500 ug/mL)	100%
Ampicillin (500 ug/mL)	＜1%
Tetracycline (500 ug/mL)	＜1%
Chloramphenicol (500 ug/mL)	＜1%

2. Interference

When 2000 ng/mL of E.coli HCP, 200 ng/mL of E.coli HCD and 100 ng/uL of plasmid DNA were added into the diluent, the recovery rates of the three samples of known concentration were 70-130%.

Cross Reactant	E.coli HCP Conc. (2000 ng/mL)			E.coli HCD Conc. (200 ng/mL)			Plasmid DNA Conc. (100 ng/uL)		
Sample Conc.(ng/mL)	5	2	0.5	5	2	0.5	5	2	0.5
Detected Sample Conc. (ng/mL)	4.57	2.00	0.400	5.09	2.20	0.58	6.06	2.40	0.62
Recovery Rate (%)	91	100	80	102	110	116	121	120	125

Robustness

When the sample binding time was between 45min-60min, the concentration CV of the three samples of known concentration were less than 20% and the recovery rate were 80-120%.

Reaction Time	45min			60min		
Samples	1	2	3	1	2	3
Sample Conc.(ng/mL)	5	2	0.5	5	2	0.5
Detected Sample Conc. (ng/mL)	4.28	2.23	0.47	4.79	1.92	0.43
CV (%)	11	8	4	3	3	11
Recovery Rate (%)	86	112	94	96	96	86

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