



User's Manual

Colistin ELISA Kit



DEIA-BY011



96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This ELISA is intended for the quantitative and qualitative analysis of colistin residue in biological samples. For research use only.

Principles of Testing

This ELISA kit is designed to detect colistin based on the principle of "indirect-competitive" enzyme immunoassay. The microtiter wells are coated with capture antigen. Colistin in the sample competes with antigen coated on the microtiter plate for the antibody. After the addition of enzyme conjugate, chromogenic substrate is used and the signal is measured by spectrophotometer. The absorption is inversely proportional to the colistin concentration in the sample.

Reagents And Materials Provided

1. Microtiter plate, pre-coated
2. Wash buffer concentrate
3. Sample diluent concentrate
4. Antibody solution, ready to use
5. Standards, ready to use
6. Spiking standard solution
7. Enzyme conjugate, ready to use
8. Substrate Solution, ready to use
9. Stop solution, ready to use

Materials Required But Not Supplied

1. Microtiter plate spectrophotometer (450nm/630nm)
2. Polystyrene centrifuge tube
3. Micropipettes
4. Deionized water

Storage

Unopened kits can be stored stably for 12 months at 2-8°C. Do not use the kit beyond the expiration date.

Assay Procedure

Notices before test:



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1. Make sure all reagents and microwells are all at room temperature (20-25°C).
2. Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the reproducibility of the ELISA analysis.
3. Avoid the light and cover the microwells during incubation.

Test procedure:

We recommend carrying out the tests in duplicate.

1. Take all reagents out at room temperature (25°C) for more than 60 min, homogenize before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. The wash solution should be brought to room temperature (20-25°C) before use.
4. Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. Add standard solution or prepared sample to corresponding wells.
6. Add enzyme conjugate solution, then antibody solution to each well, mix gently by shaking the plate manually and incubate for 40 min at 25°C with cover.
7. Remove the cover gently and pour the liquid out of the wells and rinse the microwells with diluted wash solution at interval of 10 s for 4-5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).
8. Add Substrate Solution to each well. Mix gently by shaking the plate manually, cover the plate and incubate at 25°C in dark for 15 min.
9. Add stop solution to each well. Mix gently by shaking the plate manually.
10. Read the O.D. absorbance at 450nm (recommended to use dual-wavelength 450/630nm detection) in Microplate Reader within 5min after addition of stop solution

Quality Control

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Calculation

Percentage absorbance: The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (0 ng/mL standard) and multiplied by 100%.

$$\text{Absorbance (\%)} = (B/B_0) \times 100\%$$

B - absorbance of standards or samples

B₀ - absorbance of standard 0 ng/mL