DATA SHEET

Competitive Enzyme Immunoassay Kit for Quantitative Analysis of Polymyxin B & E

1. Background

Polymyxins (polymyxin B and E) are polypeptide antibiotic, which are commonly applied in curing sensitive organism infection and promoting growth of pup and poultry. It is employed as medicine feed additives and inhibitor of Gram-negative bacteria such as bacillus pyocyaneus and Escherichia coli. Polymyxin E, also known as colistin, is excreted by kidney, and it can cause kidney epithclium denaturalization.

The common approach to detect colistin is micro- biology inhibition assay. Because of the big molecular weight, strong polarity, thermal instability, it is inappropriate to detect it with GC, thus the sensitivity and detection limit is restricted.

Enzyme linked immunoassay is more precise and sensitive, and simpler operating, and can considerably minimized work intensity.

2. Test Principle

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Colistin residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme conjugate, TMB substrate is used to show the color. Absorbance of the sample is negatively related to the colistin reside in it, after comparing with the Standard Curve, multiplied by the Dilution factor, colistin residue quantity in the sample can be calculated.

3. Applications

This kit can be used in quantitative and qualitative analysis of polymyxin B and polymyxin E (colistin) residue in vaccine and cell culture.

4. Cross-reactions

Polymyxin E (colistin).....100%
Polymyxin B.....85%
Bacitracin zinc..... < 1%

5. Materials Required

5.1 Equipments

----Microtiter plate spectrophotometer (450nm/630nm)
----Vortex mixer
----Analytical balance (inductance: 0.01g)
----Graduated pipette: 10ml
----Rubber pipette bulb
----Volumetric flask: 100ml. 1L
----Polystyrene centrifuge tubes: 2ml, 50ml
----Glass centrifuge tubes: 10ml
----Micropipettes: 20μl-200μl. 100μl-1000μl
250μl -multipipette

Reagents

----Deionized water

6. Kit Components

- Microtiter plate with 96 wells coated with antigen
- Standard solutions(6 bottles,1ml/bottle)

0ng/ml,0.5ng/ml,1.5ng/ml,4.5ng/ml,13.5ng/ml,40.5ng/ml

- Spiking standard solution : (1ml/bottle) **1μg/ml**
- Enzyme conjugate 12ml .....red cap
- Antibody solution 7ml .....green cap
- Solution A 7ml .....white cap
- Solution B 7ml .....red cap
- Stop solution 7ml .....yellow cap
- 20×concentrated wash solution 40ml
.....transparent cap
- Sample diluent 50ml.....blue cap

7. Reagents Preparation

Solution 1: Wash solution

Dilute 20×Concentrated wash solution with deionized water in the volume ratio of 1: 19, which will be used to wash the plates. This diluted solution can be stored for 1 month at 4℃.

8. Sample Preparations

8.1 Notice and precautions before operation:

(a) Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.
(b) Make sure that all experimental instruments are clean.

8.2 Vaccine & cell culture

----Dilute the sample with extraction solution to make sure

polymyxin concentration is in the range of the standard curve (0.5-40.5ng/ml).

----Take 50µl of the prepared solution for assay.

9. Assay process

9.1 Notice before assay

9.1.1 Make sure all reagents and microwells are all at room temperature (20-25℃).

9.1.2 Return all the rest reagents to 2-8℃ immediately after use.

9.1.3 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.

9.1.4 Avoid the light and cover the microwells during incubation.

9.2 Assay Steps

9.2.1 Take all reagents out at room temperature (20-25℃) for more than 30min, homogenize before use.

9.2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8℃ immediately.

9.2.3 The diluted wash solution should be rewarmed to be at room temperature before use.

9.2.4 **Number**: Numbered every microwell positions and all standards and samples should be run in duplicate. Record the standards and samples positions.

9.2.5 **Add standard/sample**: Add 50µl standard solution or prepared sample to corresponding wells. Add 50µl antibody solution. Mix gently by shaking the plate manually and incubate for 30min at 25℃ with cover.

9.2.6 **Wash**: Remove the cover gently and pure the liquid out of the wells and rinse the microwells with 250µl diluted wash solution (solution 1) at interval of 10s for 4-5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).

9.2.7 **Add enzyme conjugate**: add enzyme conjugate 100µl to each well, Mix gently by shaking the plate manually and incubate for 30min at 25℃ with cover. Repeat the wash step again.

9.2.8 **Coloration**: add 50µl solution A and 50µl solution B to each well. Mix gently by rocking the plate manually and incubate for 15min at 25℃ with cover(see 12.8)

9.2.9 **Measure**: Add 50µl stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm (It's suggested measure with the dual-wavelength of 450/630nm and read the result within 5min after addition of stop solution)

10. Results

10.1 Percentage absorbance

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbance (\%)} = \frac{B}{B0} \times 100\%$$

B ——absorbance standard (or sample)

B0 ——absorbance zero standard

10.2 Standard Curve

----To draw a standard curve: take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the polymyxin standards solution (ng/ml) as x-axis.

----The polymyxin concentration of each sample (ng/ml), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

Please notice: Special software has been developed for data analysis, which can be provided on request.

Dilution factor of samples: according to your operation.

11. Sensitivity, accuracy and precision

Test Range: 0.5 – 40.5 ng/ml

Accuracy: 80±20%

CV of the ELISA kit is less than 10%.

12. Notice

12.1 The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25℃).

12.2 Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.

12.3. Shake each reagent gently before using.

12.4. Keep your skin away from the stop solution for it is 2M H_2SO_4 solution.

12.5 Don't use the kits out of date. Don't exchange the

reagents of different batches, or else it will drop the sensitivity.

12.6 Keep the ELISA kits at 2-8℃, do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.

12.7 Substrate solution should be abandoned if it turns colors. The reagents may turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5(A450nm<0.5).

12.8 The coloration reaction need 10-15min after the addition of solution A and solution B; But you can prolong the incubation time ranges from 20min to more if the color is too light to be determined., never exceed 30min,On the contrary, shorten the incubation time properly.

12.9 The optimal reaction temperature is 25℃. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.

13. Storage condition and storage period

Storage condition: 2-8℃.

Storage period: 12 months.