Colistin ELISA Kit
DEIA042

1 Background

Colistin is a polypeptide antibiotic, which is 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. Colistin is excreted by kidney, and it can cause kidney epithclium denaturalization.
The common approach to detect colistin is microbiology inhibition assay. Due to the big molar weight, strong polarity and thermal instability, it is inappropriate to detect colistin with GC, thus the sensitivity and detection limit is restricted. Enzyme immunoassay is more precise and sensitive, and simpler operating, and can considerably minimized work intensity.

2 Test Principle

This kit is based on irect-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 Enzyme Labeled antibody. After that the 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 calculate

3 Applications

This kit can be used in quantitative and qualitative analysis of colistin in chicken and milk.

4 Cross-Reactions

Colistin……..100%
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: 500ml
----Polystyrene centrifuge tubes: 2ml, 50ml
----Glass centrifuge tubes: 10ml
----Micropipettes: 20μl-200μl. 100μl-1000μl 250μl-multipipette.

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5.2 Reagents

----Methanol (AR)
----Concentrated sulfuric acid (H2SO4, AR)
----Concentrated hydrochloric acid (HCl, AR)
----Sodium chloride (NaCl, AR)
----Deionized wate

6 Kit Components

Microtiter plate with 96 wells coated with antigen;
Standard solutions(6 bottles,1ml/bottle)
0ppb, 1.5ppb, 4.5ppb, 13.5ppb, 40.5ppb;
Enzyme conjugate(11x): 0.7ml;
Enzyme conjugate Dilution: 7ml;
Solution A 7ml;
Solution B 7ml;
Stop solution 7ml;
20×concentrated wash solution 30ml;
Extraction solution 50ml.

7 Reagents Preparation

Solution 1: Tissue extraction solution
Dilute 5ml of concentrated hydrochloric acid with deionized water to 115 ml, then add 7.5g of sodium chloride, mix completely.
Solution 2: Milk extraction solution
Dilute 2.8ml of 98% concentrated sulfuric acid with deionized water to 250ml, mix completely.
Solution 3: 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

8.1Notice 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 Tissue samples (Chicken)

----Homogenize sample with homogenizer.
----Take 1.0g±0.05g homogenized sample into a 50ml polystyrene centrifuge

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tube, add 1ml of tissue extraction solution (solution 1) and 2ml of methanol, vortex for 2min, and then centrifuge at 3000g, room temperature (20 -25℃), for 5min.
----Take 50μl of supernate, add 450μl of extraction solution **(Kit component)**, vortex for 20s to mix completely.
----Take 50μl of the prepared solution for assay

Dilution factor……40

8.3 Milk

----Take 1ml of milk to a 4ml polystyrene centrifuge tube, add 1ml of solution 2 and 1mlof methanol, vortex for 1min to mix completely.
----Centrifuge for separation: 3000g / ambient temperature/ 5min.
----Take 50μl of supernate (avonid the floating substance), add 450μl of extraction solution (**Kit component**) , vortex for 20s.
----Take 50μl of the prepared solution for assay.

Dilution factor……30

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, shake gently before use.
9.2.2 **1x Enzyme Conjugate preparation**
Dilute the enzyme conjugate with enzyme conjugate diluent in the volume ration of 1:10.
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 and enzyme conjugate:** Add 50μl of standard

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solution (**Kit component**) or prepared sample to corresponding wells. Add 50µl of 1x Enzyme conjugate. 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 3) at interval of 10s for 4 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).

9.2.7 **Coloration:** Add100µl of solution A and solution B mixture (Solution A and solution B must be thoroughly mixed according to a volume ratio of 1:1. The mixture should be used within 10 minutes. It is not allowed to use a metal container to hold or stir the reagent to avoid deterioration of the substrate.) to each well. Incubate for 15min at 25℃ with cover.

9.2.8 **Measure:** Add 50µl of stop solution(kit component) 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.

Absorbance (%)=B/B_0X100%

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 colistin standards solution (ppb) as x-axis.

----The colistin concentration of each sample (ppb), 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.

11. Sensitivity, accuracy and precision

Sensitivity: 1.5ppb

Detection Limit:

Tissue……50ppb

Milk……..60ppb

Accuracy:

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Tissue……85±15%
Milk…… 85±15%

Precision:
C.V. 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 use.
12.4 Keep your skin away from the stop solution for it is 0.5M 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 direct sunlight during all the 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 needs 10-15min after the addition of solution A and solution B. And you can prolong the incubation time ranges from 20min to more if the color is too light to be determined. Never exceed 25min, 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

Storage condition: 2-8℃.
Storage period: 12 month.

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