Version 24-01/25

User's Manual

Ceftiofur ELISA Kit

REF **DEIABL-QB56**

Σ **96T**

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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.

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PRODUCT INFORMATION

Intended Use

This kit can be used in quantitative and qualitative analysis of ceftiofur in animal tissue (pork, chicken, beef, fish and shrimp) and milk.

General Description

The ceftiofur is a kind of broad-spectrum antibiotic, which has good antimicrobial activity and characteristic of pharmacokinetics. It is effective for many gram-positive, gram-negative and bacteria producing the beta-lactamase. Ceftiofur kills the bacteria by destroying the bacterial cell wall. The popularization and application is limited because of the expensive and complicated pre-preparation and test assay.

The ELISA kit is a new product based on ELISA technology, which is fast, easy, accurate and sensitive compared with common instrumental analysis and only needs 1.5h in one run, so it can considerably minimize operation error and work intensity.

Principles of Testing

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Ceftiofur 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 ceftiofur in it, after comparing with the Standard Curve, multiplied by the dilution factor, ceftiofur quantity in the sample can be calculated.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Ceftiofur standard solutions. (1ml×6 bottles) 0 ppb, 0.5ppb, 1.5ppb, 4.5ppb, 13.5ppb, 40.5 ppb
3. Spiking standard solution: 1ml, 1ppm
4. Enzyme conjugate (12ml), red cap
5. Antibody solution (7ml), green cap
6. Solution A (7ml), white cap
7. Solution B (7ml), red cap
8. Stop solution (7ml), yellow cap
9. 20×Concentrated wash solution (40ml), transparent cap
10. 2×Concentrated extraction solution (50ml), blue cap

Materials Required But Not Supplied

1. Equipments

Microtiter plate spectrophotometer (450nm/630nm)

Rotary evaporator or nitrogen gas drying system

Shaker

Vortex mixer

Centrifuge

Analytical balance (inductance: 0.01g)

Graduated pipette: 10ml

Rubber pipette bulb

Polystyrene centrifuge tubes: 2ml, 50ml

Glass test tube: 10ml

Volumetric flask: 500ml

Micropipettes: 20μl-200μl,100μl-1000μl, 250μl-multipipette

2. Reagents

Concentrated hydrochloric acid (HCl, AR)

Acetonitrile (AR)

N-hexane (AR)

Deionized water

Storage

Storage condition: 2-8°C.

Storage period: 12 months

Specimen Collection And Preparation

Notice and precautions before operation

Please use one-off tips in the process of experiment, and change the tips when absorbing different reagent.

Make sure that all experimental instruments are clean.

Untreated sample should be stored in freeze.

Treated sample should be used immediately.

1. Tissue (pork, chicken, beef, fish and shrimp):

Take 2.0±0.05g of homogenized tissue sample into a 50ml polystyrene centrifuge tube, then add 8ml of sample extraction buffer (solution 2), shake for 5min, and then centrifuge for separation: 4000r/min / ambient temperature / 5min..

Transfer 1ml of the supernatant into a 10ml clean glass tube, dry with 50-60°C water bath under nitrogen gas stream.

Add 1ml of n-hexane, vortex for 30s, then add 1ml of extraction solution (solution 3), vortex for 30s to dissolve completely, centrifuge for separation: 4000r/min / ambient temperature / 5min..

Remove the upper organic layer, and take 50µl of the lower aqueous layer per well for assay.

Dilution factor: 4

2. Milk

Take 100µl of raw milk into 2ml centrifuge tube, and add 900µl of extraction solution (solution 3), then mix completely.

Take 50µl per well for assay.

Dilution factor: 10

Reagent Preparation

Solution 1: 0.05M HCl

Dilute 2.1ml of concentrated HCl with deionized water to 500ml.

Solution 2: Sample extraction buffer

Mix 80ml of acetonitrile with 20ml of 0.05M HCl solution.

Solution 3: Extraction solution

Dilute the 2×concentrated extraction solution with deionized water in the volume ratio of 1:1(e.g. 10ml of 2×concentrated extraction solution + 10ml of deionized water), which will be used for sample extraction, this solution can be stored at 4°C for 1 month.

Solution 4: Wash solution

Dilute the 20×concentrated wash solution with deionized water in the volume ratio of 1:19(e.g. 5ml of 20×concentrated wash solution + 95ml of deionized water), which will be used for washing the plates. This solution can be stored at 4°C for 1 month.

Assay Procedure

Notice before assay

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

Return all the rest reagents to 2-8°C immediately after used.

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.

Avoid the light and cover the microwells during incubation.

Assay Steps

1. Take all reagents out at room temperature (20-25°C) for more than 30min, 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 concentrated wash solution and concentrated extraction solution should be rewarmed to be at room temperature before use.
4. Number: Numbered every microwell positions and all standards and samples should be run in duplicate. Record the standards and samples positions.

5. Add standard solution/sample and antibody solution: Add 50µl of standard solution(Kit provided) or prepared sample to corresponding wells. Add 50µl of antibody solution(Kit provided). Mix gently by rocking the plate manually and incubate for 30min at 4-8°C with cover.
6. Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl diluted wash solution (solution 4) 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).
7. Add Enzyme conjugate: Add 100µl of enzyme conjugate(Kit provided) to each well, Mix gently by rocking the plate manually and incubate for 30min at 25°C with cover. Repeat the wash step again.
8. Coloration: Add 50µl solution A(Kit provided) and 50µl solution B(Kit provided) to each well. Mix gently by rocking the plate manually and incubate for 15min at 25°C with cover(see Precautions).
9. Measure: Add 50µl of stop solution(Kit provided) to each well. Mix gently by rocking the plate manually and measure the absorbance at 450nm (It' s suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution).

Calculation

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.

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

B ——absorbance standard (or sample)

B0 ——absorbance zero standard

2. Standard Curve

To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the ceftiofur standards solution (ppb) as x-axis.

The ceftiofur 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:

For evaluation of the result, special software has been developed, which can be provided on request.

Performance Characteristics

Accuracy

Tissue(pork, beef, chicken): 80±20%

Tissue(fish and shrimp): 80±20%

Milk: 100±20%

Precision

Variation coefficient of the ELISA kit is less than 10%

Detection Limit

Animal tissue: 2ppb

Milk: 5ppb

Sensitivity

0.5ppb

Specificity

Ceftiofur: 100%

Ceftriaxone: 169%

Cefotaxime: 63%

Cefazolin: <0.1%

Precautions

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°C).
2. Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.
3. Shake each reagent gently before use.
4. Keep your skin away from the stop solution for it is the 0.5M H_2SO_4 solution.
5. Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.
6. Keep the ELISA kits at 2-8°C,do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.
7. Substrate solution should be abandoned if it turns colors. The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5(A450nm<0.5).
8. The coloration reaction needs 15min after the addition of solution A and solution B. And you can prolong the incubation time from 20min to more if the color is too light to be determined. Never exceed 25min, on the contrary, shorten the incubation time properly.