Version 25-04/25

User's Manual

Penicillin ELISA Kit

REF **DEIABL-QB24**

Σ **96T**

RUO

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 BP residue in animal tissue (muscle, liver, shrimp and fish), milk etc.

General Description

Penicillin is a broadly applied antibiotic, whichonce played very important role in preventing and curinganimal diseases. For it causes anaphylactic reaction and resistance, in EU ,US and China, it is being restricted. The common instrumental analysis of this drug is limited because of the complicated operation and high expense, while this kit is a new product based on ELISA technology, which is fast, easy, accurate and sensitive compared with common instrumental analysis and only needs 1.5 hours in one detection, 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. BP residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme labeled anti-antibody, TMB substrate is used to show the color. Absorbance of the sample is negatively related to the BP reside in it, after comparing with the Standard Curve, multiplied by the dilution factor, BP residue quantity in the sample can be calculated.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with coupling antigen
2. Penicillin standard solutions. (1ml×6 bottles)

0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb

3. Spiking standard control: 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×Wash concentrate solution (40ml)…… transparent cap
10. 2×Concentrated extraction solution (50ml)…… blue cap

Materials Required But Not Supplied

Equipments

------Microtiter plate spectrophotometer(450nm/630nm)

------Rotary evaporator or nitrogen gas drying system

------Homogenizer

------Shaker

------Vortex mixer

------Centrifuge

------Analytical balance (inductance: 0.01g)

------Graduated pipette: 10ml

------Rubber pipette bulb

------Polystyrene centrifuge tubes: 50ml

------Glass test tube:10ml

------Volumetric flask:100ml, 500ml

------Micropipettes:20ul-200ul,200ul-10000ul,250ul-multipipette

Reagents

------Sodium hydroxide(AR)

------Acetonitrile (AR)

------n-hexane(AR)

------Deionized water

Storage

Storage condition: 2-8°C.

Storage period: 12month

Specimen Collection And Preparation

1 Notice and precautions for the users before operation

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

b. Make sure that all experimental instruments are clean.

2. Tissue sample (meat and liver of chicken, duck, fish and shrimp)

----Homogenize the samples with homogenizer;

----Weigh 2.0±0.05g of the homogenate into a 50ml polystyrene centrifuge tube, add 8ml of acetonitrile 0.1M Sodium hydroxide solution(solution 2), vortex for 2min with vortex mixer, shake for 20min with shaker, then centrifuge at room temperature (20-25°C) for 10min, at least 3000g;

----Take out 1ml of the supernate into a 10ml clean glass test tube , dry with 50-60°C water bath nitrogen gas flow;

----Dissolve the dry leftover with 1ml n-hexane, then add 1ml extraction solution (solution 3), mix completely,

centrifuge at room temperature (20-25°C) for 5min, at least 3000g;

----Remove the supernatant n-hexane phase, and dilute the substrate water phase with extraction solution(See solution 3) in the volume ratio of 1:4 (50ul extracted sample solution + 200ul extraction solution);

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

Dilution factor: 20

3. Milk

----Take 20ul of raw milk into a 50ml polystyrene centrifuge tube,

----Add 380ul of extraction solution (Solution 3), vortex for 1min to mix thoroughly.

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

Dilution factor: 20

Reagent Preparation

Solution 1: 0.1M Sodium hydroxide solution

Dissolve 0.4g of Sodium hydroxide with deionized water and dilute to 100ml;

Solution 2: Acetonitrile-0.1M Sodium hydroxide solution

Take 84ml of acetonitrile and mix with 16ml of 0.1M Sodium hydroxide solution ;

Solution 3: Extraction solution

Dilute 2×concentrated extraction solution with deionized water in the volume ration 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 for 1 month at 4°C .

Solution 4: Wash solution

Dilute 20×Concentrated wash solution with deionized water in the volume ratio of 1: 19(e.g. 5ml of 2×concentrated wash solution + 95ml of deionized water), which will be used to wash the plates. This diluted solution can be stored for 1 month at 4°C.

Assay Procedure

1. Notice before assay

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

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

c.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.

d.Avoid the light and cover the microwells during incubation.

2. Assay Steps

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

b.Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.

c.The diluted wash solution(See solution 4) should be rewarmed to be at room temperature before use.

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

e.Add standard solution/sample and antibody: Add 50ul of standard solution(kit components) or prepared sample to corresponding wells. Add 50ul antibody solution (kit components). Mix gently by rocking the plate manually and incubate for 30min at 37°C with cover.

f.Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250ul of 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).

g.Enzyme conjugate: Add100ul of enzyme conjugate (kit components) to each well, Mix gently by rocking the plate manually and incubate for 30min at 37°C with cover.

Repeat the wash step again.

h. Coloration: Add 50μl of solution A(kit components) and 50ul of solution B(kit components) to each well. Mix gently by rocking the plate manually and incubate for 15 min at 37°C with cover.

i.Measure: Add 50ul of the stop solution(kit components) to each well. Mix gently by rocking the plate manually and measure the absorbance at 450nm against an air blank (It's suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution.) (We can also measure by sight without stop solution in short of the ELISA instrument).

Calculation

There are 2 different methods to determinate the results. Method 1 leads to a round estimation, and method 2 leads to definite quantity estimation. (Please notice: the absorption is inversely proportional to the BP concentration in the sample.)

1. Round estimation

We can get the range of different strengths from the compare of average absorption and standards by sight.

For example, the absorption of sample 1 is 0.110, sample 2 is 0.820, and the absorptions of BP standard solutions: 1.880 (0ppb); 1.360(0.1ppb); 0.986(0.3ppb); 0.509(0.9ppb); 0.1906(2.7ppb); 0.079(8.1ppb). So we can say the strength of diluted sample 1 is between 2.7ppb and 8.1ppb; and diluted sample 2 between 0.3 ppb to 0.9 ppb. In order to obtain the BP actually contained in a sample, the diluted sample results must be further multiplied by the corresponding Dilution factor.

2. Definite quantity estimation

a.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_0) 100%

B ——absorbance standard (or sample)

B_0 ——absorbance zero standard

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

--- The BP 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 ELISA kits special software has been developed for exact and rapid analysis. The analysissoftware can be ordered on request.

Precision

Variation coefficient of the ELISA kit is less than 10%.

Detection Limit

Tissue sample……2ppb

Milk sample……2ppb

Accuracy:

Pork, chicken, duck, fish and shrimp…….…….. 85±10%

Milk…….…….. 85±25%

Sensitivity

0.1ppb

Specificity

Penicillin…….…… 100%

Ampicillin …….…….…… 0.7%

Cloxacillin …….…… 0.2%

Dicloxacillin ……..…… 0.1%

Amoxicillin ……..……..<0.1%

Ceftiofur ……..……<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. Homogenize each reagent before using.
4. Keep your skin away from the stop solution for it is the 2M 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. Storage condition: 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. Indications for the reagents going bad: Substrate solution should be abandoned if it turnscolors. 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 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.
9. The optimal reaction temperature is 37°C. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.