Version 16-11/24

User's Manual

Benzyl penicillin ELISA Kit

REF **DEIABL-QB25**

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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This kit can be used in quantitative and qualitative analysis of BP residue in honey, milk, milk powder, ice cream, cream etc.

General Description

Benzyl penicillin (BP) is a broadly applied antibiotic, which once played very important role in preventing and curing animal 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. Standard solutions. (1ml×6 bottles): 0 ppb, 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×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

Homogenizer

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

Sodium hydroxide (NaOH, AR)

Acetonitrile (AR)

Methanol (AR)

N-hexane (AR)

Concentrated sulfuric acid (H_2SO_4, for honey)

Concentrated hydrochloric acid (HCl, for honey)

Deionized water

Storage

Storage condition: 2-8°C.

Storage period: 12 months.

Specimen Collection And Preparation

Notice and precautions for the users 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.

Treated sample should be used immediately.

1. Milk

Take 20μl of raw milk into a 2ml polystyrene centrifuge tube,

Add 380μl of extraction solution (Solution 5), vortex for 1min to mix thoroughly.

Take 50µl of the prepared solution for assay.

Dilution factor: 20

2. Honey

Weigh 4.0±0.05g of the honey sample into a 50ml polystyrene centrifuge tube, add 0.5ml of 1M NaOH solution (solution 4), vortex completely and put it keep still for 20min.

Add 0.5ml 1M HCl solution (solution 3), vortex completely (PH should be around 3. If not, please adjust with the HCl or NaOH). Add 7ml acidified acetonitrile solution (solution 2, PH should be around 4.0). Vortex for 10min.

Centrifuge at room temperature (20-25°C) for 5min, at least 4000r/min;

Take 3ml supernatant into 10ml clean dry tube. Dry with 50-60°C water bath nitrogen gas flow;

Add 1ml extraction solution (solution 5) and vortex for 1min.

Take 50µl of the prepared solution for assay.

Dilution factor: 1

3. Milk powder

Take 1.0±0.05g of milk powder into 50ml polystyrene centrifuge tube.

Add 5ml deionized water and vortex completely till the milk powder dissolve.

Take 50µl sample solution and add 350µl extraction solution (solution 5) and vortex for 30s.

Take 50µl for assay.

Dilution factor: 40

4. Ice cream, Cream

Take 1.0±0.05g ice cream, cream into 50ml polystyrene centrifuge tube.

Add 1ml of methanol and vortex completely. Add 4ml extraction solution (solution 5) and vortex for 5s.

Take 200µl sample solution into 200µl extraction solution (solution 5) and vortex for 5s.

Take 50µl for assay.

Dilution factor: 10

Reagent Preparation

Solution 1: 2M H_2SO_4 solution (for honey)

Dissolve 10ml 98.3% Concentrated sulfuric acid with deionized water to 100ml.

Solution 2: Acidified acetonitrile solution (for honey)

Add 100ml acetonitrile into 150ul of 2M H_{24} and mix completely.

Solution 3: 1M HCl solution (for honey)

Add 41.5ml of concentrated HCl solution into deionized water to 500ml.

Solution 4: 1M Sodium hydroxide solution (NaOH, for honey)

Dissolve 4.0g of Sodium hydroxide with 100 ml of deionized water .

Solution 5: 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 6: 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

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 components) or prepared sample to corresponding wells. Add 50µl antibody solution (kit components). Mix gently by rocking the plate manually and incubate for 30min at 37°C with cover.
6. Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl of diluted wash solution (solution 6) 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. Enzyme conjugate: Add 100µl 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 6 again.
8. Coloration: Add 50µl of solution A(kit components), add 50µl of solution B(kit components) to each well. Mix gently by rocking the plate manually and incubate for 15min at 37°C with cover(see Precautions).
9. Measure: Add 50µl of the stop solution(kit components) to each well. Mix gently by rocking the plate manually and measure the absorbance at 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 ELIASA instrument).

Calculation

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

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 analysis software can be ordered on request.

Performance Characteristics

Accuracy:

Honey: 90±20%

Milk, Milk powder, Ice cream, Cream: 85±25%

Precision

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

Detection Limit

Honey: 0.1ppb

Milk: 2ppb

Milk powder: 4ppb

Ice cream, Cream: 1ppb

Sensitivity

0.1ppb

Specificity

Benzyl penicillin (BP): 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 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 need 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 25min, 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.