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User's Manual

Azithromycin ELISA Kit

REF **DEIANS005**

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 azithromycin residue in Tissue (chicken, duck).

General Description

The kit is a new product based on ELISA technology, which is fast, easy, accurate and sensitive compared with common instrumental analysis and only needs 45min 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. Azithromycin 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 azithromycin residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, azithromycin residue quantity in the sample can be calculated.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solutions. (1 ml×6 bottles)
   0 ppb, 0.075 ppb, 0.225 ppb, 0.675 ppb, 2.025 ppb, 6.075 ppb
3. Spiking standard control: 1 ml, 100 ppb
4. Concentrated Enzyme conjugate (1 ml), red cap
5. Enzyme conjugate diluent (10 ml), green cap
6. Solution A (7 ml), white cap
7. Solution B (7 ml), red cap
8. Stop solution (7 ml), yellow cap
9. 20×concentrated wash solution (40 ml), transparent cap
10. Sample Extraction solution(50 ml), blue cap
11. Sample buffer solution (3 ml), red cap

Materials Required But Not Supplied

1. Equipments

Microtiter plate spectrophotometer (450 nm/630 nm)

Rotary evaporator or nitrogen gas drying system

Homogenizer

Shaker

Centrifuge

Analytical balance (inductance: 0.01 g)

Volumetric flask: 500 ml

Polystyrene centrifuge tube: 10ml,50ml

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

2. Reagents

N-hexane(AR)

Anhydrous sodium carbonate(AR)

ethyl acetate (AR)

Sodium bicarbonate ($NaHCO_3$, AR)

Deionized water

Storage

Storage condition: 2-8°C.

Storage period: 12 months

Specimen Collection And Preparation

1. Notice and precautions for 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.

c. Treated sample can' t be stored.

2. Tissue sample(chicken, duck) (high detection limit)

a. Homogenize tissue samples with a homogenizer.

b. Weigh 2.0±0.05 g homogenized sample to 50 ml Polystyrene centrifugal tube.

c. Add 2 ml of 0.1M CB solution (solution 1), add 6 ml of ethyl acetate. shake for 3 min, then centrifuge at room temperature (20-25°C) for 5 min, at least 3000 g;

d. Remove 3 ml supernatant organic phase to 10 ml centrifuge tube, flow dry in nitrogen blow and water bath (50-60°C).

e. Add 1 ml n-hexane, vortex for 30 s, add 0.5 ml sample extraction solution, vortex for 30 s, mix completely ,then centrifuge at room temperature (20-25°C) for 5min,at least 3000 g;

f. Remove the upper organic phase, take 50 µl of the lower aqueous phase for assay.

3. Tissue sample(chicken, duck) (low detection limit)

a. Homogenize tissue samples with a homogenizer.

b. Weigh 1.0±0.05 g homogenized sample to 10 ml Polystyrene centrifugal tube.

c. Add 4 ml of deionized water, add 20ul of sample buffer solution. shake for 3 min, then centrifuge at room temperature (20-25°C) for 5 min, at least 3000 g;

d. Remove 100 µl supernatant, add 300 µl of sample extraction soution, vortex for 30 s.

e. Take 50 µl for assay.

Dilution factor of samples:

Tissue(High): 0.5

Tissue(Low): 20

Reagent Preparation

Solution 1: 0.1M CB solution

Dissolve 4.66 g of Anhydrous sodium carbonate and 0.5 g of $NaHCO_3$ with deionized water and dilute to 500 ml;

Solution 2: 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°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 30 min, 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 concentrated wash solution and concentrated extraction solution 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. Dilute the concentrated enzyme conjugate with enzyme conjugate diluent in the volume of 1:10. (the mixed solution should be used immediately).

f. **Add standard solution/sample and mixed solution (Step e):** Add 50 µl of standard solution or prepared sample to corresponding wells. Add 50 µl of mixed solution to corresponding wells. mix gently by rocking the

plate manually and Incubate for 30 min at 25°C with cover.

g. **Wash:** Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250 µl diluted wash solution (solution 2) at interval of 10 s for 4-5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).

h. **Coloration:** Add 50 µl solution A and 50 µl solution B to each well. Mix gently by rocking the plate manually and incubate for 15 min at 25°C with cover(see Precaution 8).

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

B_0 ——absorbance zero standard

2. Standard Curve

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

b. The azithromycin 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.

3. Please notice:

Special software has been developed for all data interpretation, which can be provided on request.

Performance Characteristics

Accuracy:

Tissue (High): 100±20%

Tissue(Low): 90±20%

Precision

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

Detection Limit

Tissue sample (High): 0.05 ppb

Tissue sample (Low): 2 ppb

Sensitivity

0.075 ppb

Specificity

Azithromycin: 100%

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 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. Keep the ELISA kits at 2-8°C,do not freeze. Seal rest microwell plates, avoid 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 (450/630 nm) of the zero standard is less than 0.5 (A450 nm<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 20 min 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 25°C. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.