Version 17-05/24

User's Manual

Erythromycin ELISA Kit

REF **DEIABL-QB20**

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 erythromycin residue in tissue(muscle), cell supernatant, vaccine, water, honey and milk.

General Description

Erythromycin is a macrolide antibiotic, which is applied as antibacterial and anti-mycoplasma infective. Strict MRLs have been established since this drug may lead to serious side effect in certain groups. This kit is a new product for drug residual detection based on ELISA technology, which only costs 1h in each operation and can considerably minimize operation errors and work intensity compared with instrumental analysis.

Principles of Testing

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

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Erythromycin standard solutions. (1ml×6 bottles)
   0 ppb, 0.2ppb, 0.6ppb, 1.8ppb, 5.4ppb, 16.2ppb
3. Spiking standard control 1ppm (1ml)
4. Enzyme conjugate (7ml)……..……..…….red cap
5. Antibody solution (7ml) ……green cap
6. Substrate solution(6ml*2)……..……..….red cap
7. Stop solution (7ml) ……..….yellow cap
8. 20× Concentrated wash solution (50ml) ……transparent cap
9. 2× Concentrated extraction solution (50ml) ……..… blue cap

Materials Required But Not Supplied

Equipment

1. Microtiter plate spectrophotometer (450nm/630nm)
2. Rotary evaporator or nitrogen drying instruments
3. Homogenizer

4. Shaker
5. Vortex mixer
6. Centrifuge
7. Analytical balance (inductance: 0.01g)
8. Polystyrene Centrifuge tubes: 1.5ml, 2ml, 50ml
9. Micropipettes: 20µl-200µl, 100µl-1000µl
10. 250ul-multipipette

Reagents

1. Sodium hydroxide (NaOH,AR)
2. Hydrochloric acid (HCl, AR)
3. Deionized water

Storage

Storage condition: 2-8°C.

Storage period: 12months.

Specimen Collection And Preparation

Notice and precautions before operation

1. Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.
2. Make sure that all experimental instruments are clean; otherwise it will affect the assay result.

Tissue(muscle)

1. Homogenize the tissue sample.
2. Weigh 1.0±0.05g of homogenate into a 50ml polystyrene centrifuge tube;
3. Add 4ml of 0.05M NaOH(solution 1), shake fiercely for 1min,
4. Centrifuge: 3000g / ambient temperature / 5min;
5. Transfer 200µl of supernate into a 2ml polystyrene centrifuge tube, add 760µl of extraction solution(solution 3) and 40µl of 0.1M HCl(solution 2), vortex for 30s to mix thoroughly.
6. Take 50µl of the prepared solution for assay.

   Dilution factor: 25

Honey/Milk

1. Weigh 1.0±0.05ml honey into a 50ml polystyrene centrifuge tube; add 9ml of extraction solution(solution 3), vortex to dissolve completely.
2. Take 50µl of the prepared solution for assay.

   Dilution factor: 10

Cell supernatant/Vaccine/Water

1. Take 50µl of the sample solution for assay directly

Dilution factor: 1

Reagent Preparation

Solution 1: 0.05M NaOH solution

Weigh 1.0g sodium hydroxide (NaOH)) dissolve with deionized water to 500ml.

Solution 2:0.1M HCl solution

Weigh 4.15ml concentrated hydrochloric acid dissolve with deionized water to 500ml.

Solution 3: Extraction solution

Dilute the 2× concentrated extraction solution with deionized water in the volume ratio of 1:1, 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, which will be used for washing the plates. This solution can be stored at 4°C for 1 month.

Assay Procedure

Notice before assay

1. Make sure all reagents and microwells are all at room temperature (20-25°C).
2. Return all the rest reagents to 2-8°C immediately after used.
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.
4. 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 diluted wash solution should be rewarmed to be at room temperature before use.
4. **Number:** Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. **Add standard solution/sample:** Add 50μl of standard solution or prepared sample to corresponding wells. Add 50μl enzyme conjugate solution and 50μl of antibody solution. Mix gently by shaking the plate manually and incubate for 40min at 37°C with cover.
6. **Wash:** Remove the cover gently and pure the liquid out of the wells and rinse the microwells with 250μl diluted wash solution (solution 9) 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. **Coloration:** Add 100μl of substrate solution to each well and incubate for 15 min at 37 °C with cover.
8. **Measure:** Add 50μl of the stop solution to each well. Mix gently by shaking 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 adding stop solution. We can also measure by sight without stop solution if there is no ELISA reader).

Calculation

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 (%) $=B/B_0 \times 100\%$

B ——absorbance standard (or sample)

B_0 ——absorbance zero standard

Typical Standard Curve

To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the Erythromycin standards solution (ppb) as x-axis. The erythromycin concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution multiple of each sample followed, and the actual concentration of sample is obtained. For data reduction of the ELISA kits, special software has been developed, which can be provided on request.

Precision

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

Detection Range

0-16.2ppb

Detection Limit

Tissue (muscle) ……5ppb

Honey……2ppb

Milk……2ppb

Cell supernatant……0.2ppb

Water……0.2ppb

Vaccine……0.2ppb

Sensitivity

Test Sensitivity: 0.2 ppb

Recovery

Tissue (muscle)……..……70-120%

Honey…….……70-120%

Milk……..……..……70-120%

Cell supernatant……..……70-120%

Water……..……..……70-120%

Vaccine……..……..……70-120%

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 2M H_2SO_4 solution.
5. Don't use the kits out of date. Don't exchange the reagents of different batches, for it will drop the sensitivity.
6. Keep the ELISA kits at 2-8°C,do not freeze. Seal rest microwell plates. Avoid straight sunlight for the standard sample and the colorless chromogenic reagent are sensitive to light.
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 optimal reaction temperature is 37°C. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.