Version 10-09/22

User's Manual

Tetracyclines ELISA Kit

REF **DEIA046**

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 tetracyclines residue in vaccine and cell culture.

General Description

Tetracyclines residue in the production of biological samples may lead to severe allergic reactions in certain groups. Thus it is strictly controlled in many countries in the world.

This kit is a new product for drug residual detection based on ELISA technology, which is rapid, easy-to-use, and sensitive, and can considerably minimize operation errors and work intensity.

Principles of Testing

This kit is based on direct-competitive ELISA technology. The microtiter wells are coated with antigen. Tetracyclines residue in the sample competes with the antigen coated on the 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 tetracyclines residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, tetracyclines residue in the sample can be calculated.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solutions(5×1ml/bottle)
   0ng/ml, 0.2ng/ml, 0.6ng/ml, 1.8ng/ml, 5.4ng/ml
3. Spiking standard solution: (1ml/bottle) 1μg/ml
4. Concentrated enzyme conjugate 1ml ……red cap
5. Enzyme diluent 10ml …… 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. Sample diluent 50ml……blue cap

Materials Required But Not Supplied

Equipments

1. Microtiter plate spectrophotometer (450nm/630nm)
2. Polystyrene centrifuge tube:2ml

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

Reagents

1. deionized water

Storage

Store the kit at 2-8 °C (36-46°F).

Storage period: 12 months.

Specimen Collection And Preparation

1. Dilute sample solution with sample diluent to proper tetracyclines concentration (0.2-5.4ng/ml) in it.
2. Take 50μl for assay.

Reagent Preparation

Solution 1: Wash solution

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

Assay Procedure

Notice and precautions for 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.

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, shake gently 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 sample diluent should be rewarmed 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. **Enzyme Conjugate:** Dilute the concentrated enzyme conjugate with enzyme diluent in the volume of 1:10.

   Notice: The mixture can't be stored. Please use immediately.

6. **Add standard solution/sample and enzyme conjugate:** Add 50µl of standard solution or prepared sample to corresponding wells, then add 50µl of enzyme conjugate. Mix gently by shaking the plate manually and incubate for 60min at 25°C with cover.
7. **Wash:** Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl diluted wash solution (see **solution 1**) 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).
8. **Coloration:** Add 50µl of solution A and 50µl of solution B to each well. Mix gently by shaking the plate manually and incubate for 15 min at 25°C with cover.
9. **Measure:** Add 50µl of the stop solution to each well. Mix gently by shaking 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

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

Typical Standard Curve

1. To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the tetracyclines standards solution (ng/ml) as x-axis.
2. The tetracyclines concentration of each sample (ng/ml), 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: software has been developed for data reduction, which can be provided upon request.

   Dilution factor of samples: according to your operation.

Precision

CV of the ELISA kit is less than 10%.

Detection Range

0.2-5.4ng/ml

Specificity

Tetracyclines……..……100%

Chlortetracycline……..178%

Minocycline……..118%

Doxycycline……..91%

oxytetracycline……37%

Recovery

90±10%

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 0.5M 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 coloration reaction needs 15min after adding Solution A and Solution B. And you can prolong the incubation time 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.