Version 26-04/25

User's Manual

Trimethoprim ELISA Kit

REF **DEIA-WZ037**

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 ELISA kit is designed to detect TMP based on the principle of "indirect-competitive" enzyme immunoassay.

General Description

Trimethoprim (TMP) is a bacteriostatic antibiotic mainly used in the prophylaxis and treatment of urinary tract infections. TMP is commonly used with other sulfonamides to enhance the effect of them. Due to the potential side effect of its residue in food of animal origin, strict MRLs have been established.

Principles of Testing

The microtiter wells are coated with capture BSA-linked TMP antigen. TMP in the sample competes with the precoated antigen for binding to the limited number of antibody. After the addition of enzyme conjugate and TMB substrate, the signal is measured with an ELISA photometer. The absorption is inversely proportional to the TMP concentration in the sample, compared with the standard curve; TMP residue in the sample can be calculated.

This kit is a new generation product for drug residue detection based on ELISA technology. It is fast, simple, accurate and sensitive, which considerably minimizes work intensity and operation error. It only needs 45min.

Reagents And Materials Provided

□ Microtiter plate with 96 wells precoated with antigen

□ Standard solutions(6 bottles:1ml/bottle)

0p b, 0.02ppb, 0.06 ppb, 0.18ppb, 0.54ppb,1.62ppb

□ Spiking standard solution: (1ml/bottle) 1ppm

□ Concentrated enzyme conjugate (1ml)……red cap

□ Antibody solution (7ml) ……green cap

□ Solution A (7ml)……white cap

□ Solution B (7ml)……red cap

□ Stop solution (7ml) ……yellow cap

□ 20×concentrated wash solution(40ml)……transparent cap

□ 3×Concentrated sample diluent (50ml)……blue cap

Materials Required But Not Supplied

----ELISA reader (450nm/630nm)

----Vortex mixer

----Homogenizer

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

----Volumetric flask: 500ml;

----Polystyrene centrifuge tube: 50ml, 2ml

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

----Trichloroacetic acid (TCA)

----Deionized water

Storage

2-8°C

Specimen Collection And Preparation

1 Notice and precautions 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 Honey

----Weigh 1.0±0.05g honey sample into a 10ml polystyrene centrifuge tube. Add 4ml 1.5% Trichloroacetic acid solution (solution 1), vortex till dissolve completely and then centrifuge for 5min, at least 3000g at room temperature (20-25°C);

----Take 100ul of the sample solution, add 900ul 1.5Xsample dilution (see soluton 2), vortex till dissolve completely.

---- And take 50ml of the solution for assay.

Sample dilution factor: 50

3. Aquatic products (fish and shrimp)

---Homogenize the sample.

----Weigh 1.0±0.05g aquatic product sample into a 50ml polystyrene centrifuge tube. Add 5ml 1.5% Trichloroacetic acid solution (solution 1), vortex for 2min till dissolve completely and then centrifuge for 5min, at least 3000g at room temperature (20-25°C);

----Take 100ul of the sample solution into 2ml polystyrene centrifuge tube, add 900ul 1.5Xsample dilution (see soluton 2), vortex for 30s.

---- And take 50ml of the solution for assay.

Sample dilution factor: 50

4. Milk (raw milk, finished milk)

----Take 1ml of milk sample into a 2ml polystyrene centrifuge tube. Add 1ml 1.5% Trichloroacetic acid solution

(solution 1), vortex for 30s and then centrifuge for 5min, at least 3000g at room temperature (20-25°C);

----Take 40ul of the supernatant add 960ul 1Xsample dilution (see soluton 3), vortex for 30s.

---- And take 50ml of the solution for assay.

Sample dilution factor: 50

Reagent Preparation

Solution 1: 1.5% Trichloroacetic acid solution

Weigh 7.5g Trichloroacetic acid, dissolve with deionized water and dilute to 500ml,mix completely .

Solution 2: 1.5Xsample diluent

Dilute 3Xconcentrated sample diluent, dissolve with deionized water in the volume ratio of 1:1. This solution can be stored for 1 month at 4°C.

Solution 3: 1Xsample diluent

Dilute 3Xconcentrated sample diluent, dissolve with deionized water in the volume ratio of 1:2. This solution can be stored for 1 month at 4°C.

Solution 4: Wash solution

Dilute the 20xconcentrated wash solution with deionized water in the volume ratio of 1:19, which will be used to rinse the plates. The diluted wash solution can be conserved for a month at 4°C.

Assay Procedure

1 Take all reagents out at room temperature (20-25°C) for more than 30min, each reagents should be shaken 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 concentrated sample diluent should be brought to 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.

6 Mix antibody solution and concentrated enzyme conjugate.

Dilute concentrated enzyme conjugate with antibody solution in the volume of 1:10,(1 concentrated enzyme conjugate and 10 antibody soluion) ,this mixture solution can't be stored, use it immediately.

7 Add mixture solution of antibody and enzyme: Add 50µl mixture solution of antibody and enzyme conjugate to each well, mix gently by shaking the plate manually and incubate for 30min at 25°C with cover.

8 washing: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl 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).

9 Coloration: Add 50µl solution A and 50µl solution B to each well. Mix gently by shaking the plate manually and incubate for 15min at 25°C with cover(see 12.8).

10 Measure: Add 50µl 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 from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

Absorbance(%)=B/B0 x 100%

B - the meand absorbance value of each standards or each samples

B0 - absorbance value of zero standard

Typical Standard Curve

----To draw a standard curve: The absobance value of standards as y-axis, semilogarithmic of the concentration of the standards (ppb) as x-axis.

----The TMP concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution rate of each sample followed, and the actual concentration of sample is obtained.

Precision

C.V. of the ELISA kit is less than 10%.

Detection Limit

Honey……..…1ppb

Aquatic products (fish and shrimp)……1ppb

Milk (raw milk and finished milk)……1ppb

Sensitivity

Test Sensitivity: 0.02ppb

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 the 0.5M H2SO4 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 straight sunlight during all incubations. Covering the microtiter plates is recommended.

7 Substrate solution should be abandoned if it turns colors. The reagents may 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 the addition of solution A and solution B. And you can prolong the incubation time to 20min or more if the color is too light to be determined. Never exceed 25min. On the contrary, shorten the incubation time properly.