8th Edition, revised in February, 2018

TMP (Trimethoprim) ELISA Kit

Catalog No: E-FS-E023

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit uses Competitive-ELISA as the method. It can detect TMP in samples, such as tissue, feed. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with coupled antigen. When samples are added into the ELISA Microtiter plate wells, the TMP in the samples will compete with coupled antigen on the solid phase supporter for sites of anti- TMP antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each Microtiter plate well, and TMB substrate is added for color development. There is a negative correlation between the OD value of samples and the concentration of AFM1. The concentration of AFM1 in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.015 ppb (ng/mL)

Reaction mode: 37℃, 45 min～15 min

Detection limit: Feed---0.8 ppb, Tissue (fish, shrimp and livestock) ---0.2 ppb

Cross-reactivity: Trimethoprim ---100%, Sulfapyridine---＜0.1%, Sulfanilamide ---＜0.1%, Sulfadiazine---＜0.1%, Sulfisoxazole---＜0.1%, Norsulfazole ---＜0.1%, Sulfamerazine---＜0.1%, Sulfadoxin---＜0.1%,

Sample recovery rate: Feed---85%±10%

Tissue (fish, shrimp and livestock)--- 85%±15%

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (0 ppb, 0.015 ppb, 0.045 ppb, 0.135 ppb, 0.405 ppb, 1.215 ppb)
HRP Conjugate	5.5 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20×Concentrated Wash Buffer	40 mL
2×Reconstitution Buffer	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other supplies required

Instruments: Microtiter plate reader, Printer, Homogenizer, Nitrogen Evaporators/Water bath, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

High-precision pipettor: Single-channel (20-200 μL, 100-1000 μL), Multi-channel (300 μL).

Reagents: Anhydrous methanol, N-hexane, NaOH, concentrated HCl.

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the microplate reader in advance (30 min), preheat the instrument, and set the testing parameters.

1. Sample pretreatment Notice:

Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. Solution preparation

Solution 1: Reconstitution Buffer

Dilute the 2×Reconstitution Buffer with deionized water. (2×Reconstitution Buffer (V): Deionized water (V)=1:1) .The Reconstitution buffer can be store at 4℃ for a month.

Solution 2: 0.1 M HCl Solution

Add 10 mL of concentrated HCl to 1200 mL of deionized water and mix fully.

Solution 3: 1 M NaOH Solution

Dissolve 4g of NaOH with 100 mL of deionized water and mix fully.

Solution 4: Wash buffer

Dilute 20×Concentrated Wash Buffer with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19).

3. Sample pretreatment procedure

3.1 Pretreatment of feed:

(1) Weigh 2±0.05 g of homogenate sample into 50 mL centrifuge tube. Add 20 mL of 0.1 M HCl, oscillate for 15 min, centrifuge at 3000 rpm for 10 min at room temperature;

(2) Take 1 mL of the supernatant to a clean 1.5 mL centrifuge tube, add 70 μL of 1 M NaOH (adjust pH to 6-8, the volume of 1 M NaOH can be adjusted according to different sample), mix fully. Centrifuge at 3000 rpm for 10 min at room temperature;

(3) Take 0.5 mL of the supernatant to another 1.5 mL centrifuge tube, add 0.5 mL of **Reconstitution buffer**, mix fully;

(4) Take 50 μL of for analysis.

Note: Sample dilution factor: 20, minimum detection dose: 0.8 ppb

3.2 Pretreatment of tissue (fish, shrimp and livestock):

(1) Weigh 2±0.05 g of homogenate sample that without fat into 50 mL centrifuge tube. Add 6 mL of anhydrous methanol and 2 mL of **N-hexane**, oscillate fully for 5 min at maximum speed.

(2) Centrifuge at 4000 rpm for 10 min at room temperature, discard the upper layer N-hexane, take 0.5 mL of lower layer liquid to a clean test glass (avoid to touch fat layer).

(3) Dry at 50-60℃ with Nitrogen Evaporators or water bath.

(4) Add 400 μL of **Reconstitution buffer** and 500 μL of N-hexane, oscillate fully for 1 min at maximum speed.

(5) Transfer the mixed solution to a 1.5 mL centrifuge tube, centrifuge at 4000 rpm for 5 min at room temperature. Discard the upper layer N-hexane, take 50 μL of lower layer liquid for analysis.

Note: Sample dilution factor: 5, minimum detection dose: 0.2 ppb

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate.**
2. **Add sample:** add 50 μL of **Standard or Sample** per well, then add 50 μL of **HRP Conjugate** to each well, cover the plate with sealer, oscillate for 5 sec to mix thoroughly, incubate for 45 min at 37℃.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 μL of **wash buffer** to each well and wash. Repeat wash procedure for 5 times, 30 sec intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Color Development:** add 50 μL of **Substrate Reagent A** to each well, and then add 50 μL of **Substrate Reagent B**. Gently oscillate for 5 sec to mix thoroughly. Incubate at 37℃ for 15 min with shading light (The reaction time may be shortened or prolonged according to the depth of the color).
5. **Stop Reaction:** add 50 μL of **stop solution** to each well, gently oscillate for 5 sec.
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Result analysis:

1. **Absorbance (%)=A/A_0×100%**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0 ppb Standard

2. **Drawing and calculation of standard curve**

   Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance value to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

   For this kit, it is more convenient to use professional analysis software for accurate and fast analysis on a large number of samples.

Notes

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. ELISA Microtiter plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit or reagents of different batches.
6. TMB (Substrate Reagent A or Substrate Reagent B) should be abandoned if it turns blue color. When OD value of standard (concentration: 0)＜0.5 unit (A450nm＜0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Storage and valid period

Store at 2~8℃ for 1 year. Avoid freeze.

Please store the opened kit at 2~8℃, protect from light and moisture. The valid period is 2 months.

Expiry date: expiration date is on the packing box.