Elabscience®

8th Edition, revised in February, 2018

TMP (Trimethoprim) ELISA Kit

Catalog No: E-FS-E020

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, urine, serum, etc. 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 TMP. During the reaction, TMP in the samples or standard competes with TMP on the solid phase supporter for sites of TMP antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of TMP. The concentration of TMP in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.03 ppb (ng/mL)

Reaction mode: 37℃, 45 min ~ 15 min

Detection limit: Feed---1.6 ppb, Tissue (fish/shrimp/meat/liver/kidney)---0.4 ppb, Serum/Urine/Plasm---0.4 ppb

Cross-reactivity: Trimethoprim ---100%, Sulfapyridine --- ＜ 0.1%, Sulfanilamide --- ＜ 0.1%, Sulfadiazine ---＜0.1%, Sulfasoxazole ---＜0.1%, Sulfathiazole ---＜0.1%, Sulfamerazine ---＜0.1%, Sulfadoxine ---＜0.1%

Sample recovery rate: Feed---95% ± 15%, Tissue (fish/shrimp/meat/liver/kidney)---95% ± 10%, Serum/urine/plasma---85%±10%

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (0 ppb, 0.03 ppb, 0.09 ppb, 0.27 ppb, 0.81 ppb, 2.43 ppb)
High Concentrated Standard (100 ppb)	1 mL
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

Other supplies required

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

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

Reagents: Absolute methanol, N-hexane, Hydrochloric acid, Sodium hydroxide.

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate reader in advance, 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 :deionized water=1:1) .The Reconstitution buffer can be store at 4℃ for a month.

Solution 2: 0.1 M Hydrochloric acid

Pipet 10 mL of concentrated Hydrochloric acid into 1200 mL deionized water.

Solution 3: 1 M Sodium hydroxide

Dissolve 4 g of Sodium hydroxide with 100 mL of deionized water

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 g of smashed sample into a 50 mL centrifugal tube, add 20 mL of 0.1 M hydrochloric acid and vibrate for 15 min. Centrifuge at 3000 r/min at room temperature for 10 min.

(2) Take 1 mL of upper liquid into a 1.5 mL centrifugal tube, adjust the pH to 6~8 with 1 M NaOH. (The added amount of 1 M NaOH is different according to the feed sample. The needed amount is generally between 70μL ~100 μL.), centrifuge at 3000 r/min at room temperature for 10 min.

(3) Take 0.5 mL of upper liquid into another 1.5 mL centrifugal tube, add 0.5 mL of Reconstitution buffer, and mix thoroughly.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 20, minimum detection dose: 1.6 ppb

3.2 Pretreatment of Tissue (fish/shrimp/meat/liver/kidney):

(1) Weigh 2 g of homogenate tissue sample (after remove the fat) into a 50mL centrifugal tube, add 6 mL of absolute methanol and 2 mL of N-hexane, whirl for 5 min with the highest speed.

(2) Centrifuge at 4000 r/min at room temperature for 10 min, remove the upper N-hexane, take 0.5 ml of lower clear liquid into clean glass test tube(do not touch the fat layer).

(3) Dry at 50-60℃ under a gentle stream of nitrogen or water bath.

(4) Add 400 μL of Reconstitution buffer and 500 μL of N-hexane, oscillate for 1 min with highest speed.

(5) Shift it to a 1.5 mL centrifugal tube, centrifuge at 4000 r/min at room temperature for 5 min, and remove upper N-hexane, take 50 μL of lower clear liquid for analysis.

Note: Sample dilution factor: 5, minimum detection dose: 0.4 ppb

3.3 Pretreatment for Urine/Serum/Plasma:

(1) Weigh 0.5 mL of sample, centrifuge at 4000 r/min at room temperature for 5 min.

(2) Take 50 μL of supernatant, add 200 μL of Reconstitution buffer r, and mix thoroughly.

(3) Take 50 μL for analysis.

Note: Sample dilution factor: 5, minimum detection dose: 0.4 ppb

(If necessary, you could increase the amount of Reconstitution solution to increase the dilution factor)

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.
2. **Add sample:** add 50 μL of standard or sample to each well, then add 50 μL of HRP Conjugate to each well, cover the plate with sealer we provided, oscillate for 5 sec gently 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, 30s 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 solution A to each well, and then add 50 μL of substrate solution B. Gently oscillate for 5 s to mix thoroughly. Incubate shading light for 15 min at 37℃.(If the blue is too shallow, the reaction time can be prolonged appropriately).
5. **Stop reaction:** add 50 μL of stop solution to each well, oscillate gently to mix thoroughly.
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. to each.

Result analysis

1. **Absorbance (%) = $A/A_0 \times 100\%$**

   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 of sample 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. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperature is below 25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor repeatability, move on to 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 and reagents of different batches of kits.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0) is below 0.5 unit (A_{450nm}＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, expiration date is on the packing box.