8th Edition, revised in February, 2018

TMP (Trimethoprim) ELISA Kit

Catalog No: E-FS-E023

96T/96T*3

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.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

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 for the quantitative detection. It can detect Trimethoprim (TMP) in samples, such as muscle, feed, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, standard liquid and other supplementary reagents. The microtiter plate in this kit has been pre-coated with antibodies. During the reaction, TMP in the samples or standard competes with Horseradish Peroxidase (HRP) conjugate for sites. Add substrate reagent is added 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

Reaction mode (Incubation time and temperature): 37℃; 45 min-15 min

Detection limit: Feed---0.8 ppb; Muscle---0.2 ppb; Serum, Urine---0.2 ppb

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

Sample recovery rate: Feed---85%±10%; Serum, Urine---85%±10%; Muscle--- 85%±15%

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (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 materials required but not supplied

Instruments: Microplate reader, Printer, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

Micropipette: Single channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).

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

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. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E023. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E023 with different lot numbers.**
6. Substrate Reagent 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 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 expiry date

Store the kit at 2~8℃. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2 - 8℃.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore all reagents and samples to room temperature before use.

Open the microplate 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 (V): Deionized water (V) =1:1). The Reconstitution buffer can be store at 4℃ for a month.

Solution 2: 0.1 M HCl Solution *(for feed sample)*

Dilute 10 mL of **Concentrated HCl** to 1200 mL with deionized water, mix fully.

Solution 3: 1 M NaOH Solution *(for feed sample)*

Dissolve 4g of **NaOH** to 100 mL with deionized water, 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

Substance in sample is distributed unevenly. It is recommended that more samples should be taken when sampling.

3.1 Pretreatment of feed sample:

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

(2) Take 1 mL of the supernatant to a clean 1.5 mL centrifuge tube, add about 70 μL of **1 M NaOH Solution** (Solution 3), adjust pH to 6-8 (the volume of 1 M NaOH can be adjusted according to different sample), mix fully. Centrifuge at 3000 r/min 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** (Solution 1) , mix fully;

(4) Take 50 μL of for analysis.

Note: Sample dilution factor: 20, detection limit: 0.8 ppb

3.2 Pretreatment of muscle (animal food commodities) sample:

(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**, vortex fully for 5 min.

(2) Centrifuge at 4000 r/min 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** ((Solution 1) and 500 μL of **N-hexane**, vortex fully for 1 min at maximum speed.

(5) Transfer the mixed solution to a 1.5 mL centrifuge tube, centrifuge at 4000 r/min for 5 min at room temperature. Discard the upper layer N-hexane.

(6) Take 50 μL of lower layer liquid for analysis.

Note: Sample dilution factor: 5, detection limit: 0.2 ppb

3.3 Pretreatment of serum, urine (swine) sample:

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

(2) Take 50 μL of supernatant, add 200 μL of **Reconstitution Buffer** (Solution 1) and mix fully.

(3) Take 50 μL for analysis.

Note: Sample dilution factor: 5, detection limit: 0.2 ppb

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2~8℃.

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 5s to mix thoroughly, incubate at 37℃ for 45 min in shading light..
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 μL of **Wash Buffer** (Solution 4) 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 Reagent A** to each well, and then add 50 μL of **Substrate Reagent B**. Gently oscillate for 5s 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 10s to mix thoroughly.
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. 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 form for accurate and fast analysis on a large number of samples.

Trimethoprim (E-FS-E023) **Standard Curve**