Technical Manual

TMP (Trimethoprim) ELISA Kit

- **Catalogue Code: FSES0015**
- **Competitive ELISA Kit**
- **Research Use Only**

Contents

1. Key features and Sample Types 3
2. Storage 3
3. Test Principle 3
4. Kit Contents 4
5. Experimental Preparation 5
6. Assay Procedure 6
7. Data Analysis 6
8. Notes 7

1. Key features and Sample Types

Sensitivity:

0.03 ppb (ng/mL)

Assay Procedure:

37°C, 45 min ~ 15 min

Detection Limit:

Feed - 1.6 ppb; Muscle, Kidney, Liver - 0.4 ppb; Serum, Urine - 0.4 ppb

Cross Reactivity:

Trimethoprim - 100%, Sulfapyridine - < 0.1%, Sulfamethoxazole - < 0.1%, Sulfisoxazole - < 0.1%, Sulfathiazole - < 0.1%, Sulfamerazine - < 0.1%, Sulfadoxine - < 0.1%

Sample Recovery rate:

Feed - 95%±15%; Muscle, Liver, Kidney - 95%±10%; Serum, Urine - 85%±10%

Storage:

2-8°C for 6 months.

Expiry:

See Kit Label

2. Storage

Store the kit at 2~8°C. 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°C.

3. Test Principle

This kit uses a Competitive-ELISA method. 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.

4. Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	96-WellKit
1	ELISA Microtiter plate	96 wells
2	Standards	1 mL each (0 ppb, 0.03 ppb, 0.09 ppb, 0.27 ppb, 0.81 ppb, 2.43 ppb)
3	HRP Conjugate	5.5 mL
4	Substrate Reagent A	6 mL
5	Substrate Reagent B	6 mL
6	Stop Solution	6 mL
7	20×Concentrated Wash Buffer	40 mL
8	2×Reconstitution Buffer	50 mL
9	Plate Sealer	3 pieces
10	Sealed Bag	1 piece
11	Manual	1 copy

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

Additional materials required:

Other materials required but not supplied

- **Instruments:** Microplate reader, Printer, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensitivity 0.01 g).
- **Micropipette:** Single channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).
- **Reagents:** Absolute methanol, N-hexane, Concentrated HCl, NaOH.

5. 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 pre-treatment 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 **2×Reconstitution Buffer** with deionized water (**2×Reconstitution Buffer** (V): Deionized water (V) = 1:1).

Solution 2: 0.1 M HCl Solution (for feed sample)
Dilute 10 mL of **Concentrated HCl** to 1200 mL with deionized water.

Solution 3: 1 M NaOH Solution (for feed sample)
Dissolve 4 g of **NaOH** to 100 mL with 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 pre-treatment procedure

Targets may be distributed unevenly, resulting in no detection. To avoid this, ensure to take sufficient samples when sampling.

3.1 Pre-treatment of feed sample:

1. Weigh 2 g of homogenate sample into a 50 mL centrifugal tube, add 20 mL of **0.1 M HCl Solution**
   (Solution 2) and vortex for 15 min. Centrifuge at 3000 r/min at room temperature for 10 min.
2. Take 1 mL of the supernatant into 1.5 mL centrifugal tube, adjust the pH to 6~8 with **1 M NaOH Solution** (Solution 3) (The added amount of 1 M NaOH Solution 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 the supernatant into another 1.5 mL centrifugal tube, add 0.5 mL of **Reconstitution Buffer** (Solution 1), and mix thoroughly.
4. Take 50 μL for analysis.
   Note: Sample dilution factor: 20, detection limit: 1.6 ppb

3.2 Pre-treatment of muscle, liver, kidney sample:

1. Weigh 2 g of homogenate sample (after remove the fat) into a 50mL centrifugal tube, add 6 mL of **Absolute methanol** and 2 mL of **N-hexane**, vortex for 5 min.
2. Centrifuge at 4000 r/min at room temperature for 10 min, remove the upper N-hexane layer, take
   0.5 mL of lower liquid into clean glass test tube (do not touch the fat layer) and dry at 50-60°C with nitrogen evaporators or water bath.
3. Add 400 μL of **Reconstitution Buffer** (Solution 1) and 500 μL of **N-hexane**, vortex

for 1 min. Centrifuge at 4000 r/min at room temperature for 5 min.

4. Remove upper N-hexane layer, take 50 µL of lower liquid for analysis.
   Note: Sample dilution factor: 5, detection limit: 0.4 ppb

3.3 Pre- treatment for urine, serum (swine)* sample:

***Data validated in swine urine but pre-treatment can be applied for urine samples of multiple species.**

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** (Solution 1), and mix thoroughly.
3. Take 50 µL for analysis.
   Note: Sample dilution factor: 5, detection limit: 0.4 ppb

6. Assay Procedure

Bring all reagents and samples to room temperature 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°C.

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** to each well, then add 50 µL of **HRP Conjugate** to each well, cover the plate with plate sealer, vortex for 5s gently to mix thoroughly, incubate at 37°C for 45 min away from direct sunlight.
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 absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Colour Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50 µL of **Substrate Reagent B**. Gently vortex for 5s to mix thoroughly. Incubate at 37°C for 15 min away from direct sunlight. (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, vortex gently 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.

7. Data 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 form for accurate and fast analysis on a large number of samples.

8. 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°C.
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 kit to strong light.
5. **Each reagent is optimized for use in the FSES0015. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other FSES0015 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue colour. When OD value of standard (concentration: 0) < 0.5 unit (A450nm < 0.5), it indicates the reagents are 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.

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