SMZ (Sulfamethoxazole) ELISA Kit
Catalog No: E-FS-E021
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 Indirect-Competitive-ELISA as the method. It can detect Sulfamethoxazole (SMZ) in samples, such as tissue, serum, honey, fish milk and urine, etc. This kit is composed of ELISA Microtiter plate, conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with SMZ. SMZ in the samples or standard competes with SMZ on the solid phase supporter for sites of SMZ antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro 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 SMZ. The concentration of SMZ in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator
Sensitivity: 0.1 ppb (ng/mL)
Reaction mode: 25℃, 45 min~15 min
Reaction rate: Sulfamethoxazole---100%
Detection limit: Tissue (high detection limit method) ---0.1 ppb, Tissue (low detection limit method) ---1 ppb, Honey---0.1 ppb, Serum/Urine/Egg---0.4 ppb, Milk---2 ppb, Feed---4 ppb
Sample recovery rate: Tissue/Honey/Egg ---85% ± 25%, Serum/Urine/Milk/Feed---80% ± 25%

Kits components

<table>
<thead>
<tr>
<th>Item</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA Microtiter plate</td>
<td>96 wells</td>
</tr>
<tr>
<td>Standard Liquid</td>
<td>1 mL each (0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb)</td>
</tr>
<tr>
<td>High Concentrated Standard(1 ppm)</td>
<td>1 mL</td>
</tr>
<tr>
<td>HRP Conjugate</td>
<td>5.5 mL</td>
</tr>
<tr>
<td>Antibody Working Solution</td>
<td>5.5 mL</td>
</tr>
<tr>
<td>Substrate Reagent A</td>
<td>6 mL</td>
</tr>
<tr>
<td>Substrate Reagent B</td>
<td>6 mL</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>6 mL</td>
</tr>
<tr>
<td>20×Concentrated Wash Buffer</td>
<td>40 mL</td>
</tr>
<tr>
<td>2×Reconstitution Buffer</td>
<td>50 mL</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td>3 pieces</td>
</tr>
<tr>
<td>Sealed Bag</td>
<td>1 piece</td>
</tr>
<tr>
<td>Manual</td>
<td>1 copy</td>
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</tbody>
</table>
Other supplies required

**Instruments:** Microplate reader, Printer, Homogenizer, Nitrogen Evaporators/Water bath, Oscillators, Centrifuge, Graduated pipette, Balance (sensitivity 0.01 g).

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

**Reagents:** Ethyl acetate, Concentrated (HCl), N-hexane, Acetonitrile, \( \text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O} \), \( \text{NaOH} \), \( \text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O} \).

**Experimental preparation**

1. **Sample pretreatment Notice:**

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

2. **Solution preparation**

   Solution 1: 0.1 M PBS Buffer

   Dissolve 25.8 g of \( \text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O} \) and 4.4 g of \( \text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O} \) with 1000 mL deionized water.

   Solution 2: Acetonitrile-ethyl acetate Solution

   Add 50 mL of Acetonitrile and 50 mL of Ethyl acetate to 100 mL glass bottle, mix fully.

   Solution 3: 0.5 M HCl Solution

   Add 4.3 mL of Concentrated (HCl) to 100mL of deionized water, mix fully.

   Solution 4: 0.2 M NaOH Solution

   Dissolve 0.8 g of NaOH with 100 mL deionized water.

   Solution 5: 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°C for a month.

   Solution 6: 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 tissue sample (High detection limit, method 1):

(1) Weigh $2 \pm 0.05$ g of homogenate sample into 50 mL EP tube. Add 1 mL of 0.1 M PBS, Oscillate the sample into a paste with a vortex. Add 7 mL of Acetonitrile-ethyl acetate solution, Oscillate for 2 min, centrifuge at 4000 r/min for 10 min at room temperature.

(2) Take 4 mL of the clean organic layer to a dry container, dry at 50-60°C under a gentle stream of nitrogen or water bath.

(3) Redissolve the dry residual sediment with 1 mL of N-hexane. Add 1 mL of Reconstitution buffer and mix for 30 sec. Centrifuge at a speed over 4000 r/min for 5 min at room temperature.

(4) Remove the upper layer, and take 50 μL of the lower layer for analysis.

Note: Sample dilution factor: 1, minimum detection dose: 0.1 ppb

3.2 Pretreatment of tissue sample (Low detection limit):

(1) Weigh $1 \pm 0.05$ g of homogenate into a 50 mL EP tube, add 9 mL of 0.1 M PBS Buffer and oscillate for 5 min, centrifuge at a speed over 4000 r/min for 5 min at room temperature.

(2) Take 50 μL of the supernatant for analysis.

Note: Sample dilution factor: 10, minimum detection dose: 1 ppb

3.3 Pretreatment of eggs sample

(1) Use homogenizer to homogenize egg sample, so that egg white and egg yolk fully mixed.

(2) Weigh $2 \pm 0.05$ g of homogenate sample into 50 mL EP tube. Add 8 mL of 0.1 M PBS Buffer and oscillate fully for 30 sec. Centrifuge at a speed over 4000 r/min for 5 min at room temperature.

(3) Take 1 mL of the supernatant to 10 mL clean dry glass, dry at 50-60°C under a gentle stream of nitrogen or water bath.

(4) Redissolve the dry residual sediment with 1 mL of N-hexane. Add 1 mL of Reconstitution buffer and mix for 30 sec. Centrifuge at a speed over 4000 r/min for 5 min at room temperature. Discard the upper layer solution.

(5) Take 50 μL of the lower later solution for analysis.

Note: Sample dilution factor: 4, minimum detection dose: 0.4 ppb

3.4 Pretreatment of serum sample:

(1) Stand the serum for 30 min at room temperature. Centrifuge at a speed above 4000 r/min for 10 min at room temperature, after the serum separated out.

(2) Take 1 mL of serum sample. Add 3 mL of 0.1 M PBS Buffer and oscillate fully for 30 sec.

(3) Take 50 μL for analysis.

Note: Sample dilution factor: 4, minimum detection dose: 0.4 ppb
3.5 Pretreatment of honey sample:
(1) Weigh 1 ± 0.05 g of honey sample into a 50 mL EP tube. Add 1 mL of 0.5 M HCl. Incubate for 30 min at 37℃.
(2) Add 2.5 mL of 0.2 M NaOH (pH≈5), then add 4mL Ethyl acetate. Oscillate for 5 min, centrifuge at a speed over 4000 r/min for 5 min at room temperature.
(3) Take 2 mL of the upper layer solution to a dry container, dry at 50-60℃ under a gentle stream of nitrogen or water bath.
(4) Redissolve the dry residual sediment with 0.5 mL of Reconstitution buffer. Mix for 30 sec.
(5) Take 50 μL for analysis.
   Note: Sample dilution factor: 1, minimum detection dose: 0.1 ppb

3.6 Pretreatment of urine sample:
(1) Add 3 mL of 0.1 M PBS Buffer into 1mL of centrifuged clear urine sample, oscillate for 30 sec.
(2) Take 50 μL for detection and analysis.
   Note: Sample dilution factor: 4, minimum detection dose: 0.4 ppb

3.7 Pretreatment of milk sample:
(1) Dilute 100 μL of milk with 0.1 M PBS Buffer (1:19, v/v, 100μL of milk +1.9 mL of 0.1M PBS Buffer). Mix for 30 sec.
(2) Take 50 μL for analysis.
   Note: Sample dilution factor: 20, minimum detection dose: 2 ppb

3.8 Pretreatment of feed sample:
(1) Weigh 2.0±0.05 g of feed sample into 50 mL polystyrene centrifuge tube, add 8 mL of acetonitrile, oscillate 5 min, centrifuge at a speed over 4000 r/min for 5 min at room temperature.
(2) Take 1 mL of the upper organic layer to 10 mL clean dry glass, blow-dry at 50-60℃ under a gentle stream of nitrogen or water bath.
(3) Add 1 mL of N-hexane, use vortex to vortex sample for 30 sec, then add 1 mL of 0.1 M PBS Buffer, vortex sample for 30 sec, transfer sample to 2 mL polystyrene centrifuge tube, centrifuge at a speed over 4000 r/min for 5 min at room temperature.
(4) Remove the upper organic layer, take 100 μL of the lower water layer to 2 mL EP tube, add 900 μL of 0.1 M PBS Buffer, vortex sample for 1 min, mix well;
(5) Take 50 μL sample for analysis.
   Note: Sample dilution factor: 40, minimum detection dose: 4 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 wells), and keep a record of standard wells and sample wells.

2. **Add sample:** Add 50 μL of Standard or Sample per well, then add 50 μL of HRP Conjugate to each well. Add 50 μL of antibody working solution. Gently oscillate for 5 sec to mix thoroughly and cover the plate with sealer. Incubate for 45 min at 25°C.

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 solution A to each well, and then add 50 μL of substrate solution B. Gently oscillate for 5 sec to mix thoroughly. Incubate shading light for 15 min at 25°C in the dark. (If the blue color is too shallow, can extend the incubation time properly.

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.

Result analysis
1. **Absorbance (%) =A/A₀×100%**  
   A: Average absorbance of standard or sample  
   A₀: 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°C.

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)<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°C. Avoid freeze / thaw cycles.

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