Elabscience®

7th Edition, revised in April, 2017

（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

SMD (Sulfametoxydiazine) ELISA Kit

Catalog No: E-FS-E052

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) 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 SMD in samples, such as tissue, serum, honey, milk, urine, etc. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with SMD. During the reaction, SMD in the samples or standard competes with SMD on the solid phase supporter for sites of SMD 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 SMD. The concentration of SMD in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.05 ppb (ng/mL)

Reaction mode: 25℃, 45 min~ 15 min

Detection limit: Tissue (high detection limit) ---0.5 ppb,
Tissue (low detection limit) ---0.25 ppb,
Serum/urine---0.2 ppb,
Honey---0.05 ppb,
Milk---1 ppb.

Cross-reactivity:

Names	Cross-reactivity
Sulfametoxydiazine(SMD)	100%
Sulfadiazine (SD or SDZ)	40%
Sulfamerazine(SM1)	25%
Sulfadoxine (SDM')	35%

Sample recovery rate: Tissue/honey---95%±25%, Urine/milk/serum ---85%±25%

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Liquid (black cap)	1mL each (0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb)
HRP Conjugate (red cap)	5.5 mL
Antibody Working Solution (blue cap)	5.5 mL
Substrate Reagent A (white cap)	6 mL
Substrate Reagent B (black cap)	6 mL
Stop Solution (yellow cap)	6 mL
20×Concentrated Wash Buffer (white cap)	40 mL
2×Reconstitution Buffer (yellow cap)	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Other supplies required

Instruments: Microplate reader, Printer, Homogenizer, Nitrogen Evaporators, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01g).

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

Reagents: Ethyl acetate, n-hexane, dichloromethane, acetonitrile, $Na_2HPO_4 \cdot 12H_2O$, NaOH, Concentrated HCl, $NaH_2PO_4 \cdot 2H_2O$.

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: 0.2 M NaOH Solution

Dissolve 0.8 g of NaOH to 100 mL of deionized water

Solution 2: 0.02 M PB Buffer

Dissolve 2.58 g of $Na_2HPO_4 \cdot 12H_2O$ and 0.44 g of $NaH_2PO_4 \cdot 2H_2O$ to 500 mL of deionized water

Solution 3: 0.5 M HCl Solution

Add 4.3 mL of concentrated HCl into 100mL of deionized water and mix thoroughly

Solution 4: 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 5: 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 (high detection limit):

(1) Add 3±0.05 g of homogeneous tissue sample to a centrifuge tube, then add 3 mL of 0.02 M PB buffer solution, oscillate and mix thoroughly. Add 4 ml of ethyl acetate and 2 mL of acetonitrile, oscillate for 10 min, centrifuge at above 4000 r/min for 10 min.

(2) Take 2 mL of upper liquid (about 1 g of sample), blow-dry at 50-60℃ with nitrogen or air.

(3) Dissolve the residual with 1 mL of n-hexane, add 1 mL of Reconstitution buffer and oscillate for 1 min. Centrifuge at 4000 r/min for 5 min.

(4) Discard the upper n-hexane, take 50 μL of lower liquid for analysis.

Note: Sample dilution factor: 1, minimum detection dose: 0.05 ppb

3.2 Pretreatment of tissue (low detection limit):

(1) Add 2.0±0.05 g of homogeneous tissue sample to a centrifuge tube, then add 8 mL of 0.02 M PB buffer solution, oscillate for 2 min, centrifuge at above 4000 r/min for 10 min.

(2) Take 50 μL of liquid for analysis.

Note: Sample dilution factor: 5, minimum detection dose: 0.25 ppb

3.3 Pretreatment of serum:

(1) Put blood sample at room temperature for 30 min, centrifuge at above 4000 r/min for 10 min, separate the serum or filter the serum.

(2) Take 1 mL of serum, add 3 mL of 0.02 M PB buffer solution, and mix for 30 sec.

(3) Take 50 μL of liquid for analysis.

Note: Sample dilution factor: 4, minimum detection dose: 0.2 ppb

3.4 Pretreatment of honey:

(1) Weigh 1±0.05 g of honey sample into 50 mL a centrifuge tube, add 1 mL of 0.5 M HCl solution, put at 37℃ for 30 min.

(2) Add 2.5 mL of 0.2 M NaOH solution (adjust the PH value to about 5), then add 4 mL of ethyl acetate, oscillate for 5 min, centrifuge at above 4000 r/min at room temperature for 10 min.

(3) Take 2 mL of upper liquid, blow-dry at 50-60℃ with nitrogen or air. Add 0.5 mL of Reconstitution buffer and mix for 30 sec.

(4) Take 50 μL of liquid for analysis.

Note: Sample dilution factor: 1, minimum detection dose: 0.05 ppb

3.5 Pretreatment of urine:

(1) Mix 3 mL of 0.02 M PB buffer solution and 1 mL of centrifuged clear urine sample for 30 sec.

(2) Take 50 μL of liquid to analysis.

Note: Sample dilution factor: 4, minimum detection dose: 0.2 ppb

3.6 Pretreatment of milk:

(1) Dilute milk sample with 0.02 M PB buffer solution with the ratio of 1:20 (for example, 20 μL milk + 380 μL of 0.02 M PB buffer), mix for 30 sec.

(2) Take 50 μL of liquid to analysis.

Note: Sample dilution factor: 20, minimum detection dose: 1 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.
2. **Add sample:** add 50 μL of Standard or Sample per well, then add 50μL of HRP Conjugate to each well, then add 50 μL of antibody working solution, cover the plate with sealer we provided, oscillate for 5 sec gently to mix thoroughly, incubate for 45 min at 25℃.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 250 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℃ (The reaction time can be extended according to the actual color change).
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 micro-plate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Result analysis

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

   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) Micro ELISA 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, production date is on the packing box.