Elabscience®

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SUD (Sudan Red) ELISA Kit

Catalog No: E-FS-E016

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)
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Please kindly provide us the lot number(on the outside of the box) of the kit for more efficient service.

Test principle and application

This kit uses Indirect-Competitive-ELISA as the method. It can detect SUD in samples, such as tomato sauce, chilli sauce, eggs, 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 SUD. During the reaction, SUD in the samples or standard competes with SUD on the solid phase supporter for sites of SUD 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 SUD. The concentration of SUD in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.3 ppb (ng/mL)

Reaction mode: 25℃, 30min~ 30min~15min

Detection limit: Tomato juice/tomato sauce/ chilli sauce---12ppb,
Chilli powder (Pepper noodles)/fodder---120ppb,
Eggs (Chicken egg,Duck egg,Goose egg) ---30ppb

Cross-reactivity: SUD---100%, Para Red ---123%, Rhodamine ---8%,

Sample recovery rate: Tomato juice/tomato sauce/ chilli sauce ---80%±15%,
Chilli powder(Pepper noodles)/fodder ---95%±15%,
Eggs(Chicken egg, Duck egg, Goose egg) ---80%±15%

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
High Concentrated Standard	1 mL
Standard Liquid (black cap)	1 mL each 0ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb
HRP Conjugate (red cap)	11 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
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Other supplies required

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

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

Reagent: Methanol.

Experimental preparation

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: 10% Methyl alcohol

Add 10mL Methyl alcohol to 90 mL deionized water, mix fully, closed spare

Solution 2: 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 Tomato juice/tomato sauce/ chilli sauce:

(1) Weigh 2±0.05 g of homogenate sample into centrifuge tube, add 10 mL of Methyl alcohol, oscillate for 5 min, centrifuge at 4000 r/min at room temperature for 10 min.
(2) Take 100 μL of upper liquid to mix with 700 μL of deionized water.
(3) Take 50 μL of the mixture to analyze.

Note: Sample dilution factor: 40, minimum detection dose: 12 ppb

3.2 Pretreatment of Chilli powder/fodder:

(1) Weigh 1±0.05 g of sample into centrifuge tube, add 10mL of Methyl alcohol, oscillate for 5 min, centrifuge at 4000 r/min at room temperature for 10 min.
(2) Take 20 μL of upper liquid to mix with 780 μL of 10% Methyl alcohol.
(3) Take 50 μL of the mixture to analyze.

Note: Sample dilution factor: 400, minimum detection dose: 120 ppb

3.3 Pretreatment of eggs:

(1) Homogeneous egg samples with Homogenizer at low-speed (Cooked egg sampling egg yolk, raw egg sampling whole egg)

(2) Weigh 1±0.05 g of homogenate eggs sample into centrifuge tube, add 9 mL of Methyl alcohol , oscillate for 5 min (oscillate violently to separate a group of samples, mixed fully), centrifuge at 4000r/min at 15℃ for 10 min.

(3) Take 100 μL of upper liquid, add 900 μL of deionized water, mixed fully.

(4) Take 50 μL of the mixture to analyze.

Note: Sample dilution factor: 100, minimum detection dose: 30 ppb

If the sample serious pollution, more impurities or residues seriously exceeded, it should be further diluted after reanalysis.

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.

Before the experiment to be prepared standard solution. As low concentration of the standard solution is instable, it need be use right after it was ready. Then make serial dilution as follows:

(1) Take 3 mL of 10% Methanol (solution 1) into **0 ppb bottle**. Take 2 mL of 10% Methanol into **0.3 ppb bottle**, **0.9 ppb bottle**, **2.7 ppb bottle, 8.1 ppb bottle** respectively. Take 2.93 mL of 10% Methanol into **24.3 ppb bottle**.

(2) **Standard Solution 6**: Take 73 μL of 1.0 ppm high concentrated standard into 24.3 ppb bottle, then mix fully. The concentration of Standard Solution 6 is 24.3 ppb.

(3) **Standard Solution 5**: Take 1 mL of Standard Solution 6 into 8.1 ppb bottle, then mix fully. The concentration of Standard Solution 5 is 8.1 ppb.

(4) **Standard Solution 4**: Take 1 mL of Standard Solution 5 into 2.7 ppb bottle, then mix fully. The concentration of Standard Solution 4 is 2.7 ppb.

(5) **Standard Solution 3**: Take 1 mL of Standard Solution 4 into 0.9 ppb bottle, then mix fully. The concentration of Standard Solution 3 is 0.9 ppb.

(6) **Standard Solution 2**: Take 1 mL of Standard Solution 3 into 0.3 ppb bottle, then mix fully. The concentration of Standard Solution 2 is 0.3 ppb.

(7) **Standard Solution 1**: Use 10% Methanol directly. The concentration of Standard Solution 1 is 0ppb.

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 50 μL 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℃.
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/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 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.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)＜0.5 unit(A450nm＜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.