



Data Sheet

Sudan Red (SUD) ELISA Kit

Cat. #ELAB53

Size: 96 Wells



Principle and Application

This kit adopts the method of indirect competitive enzyme-linked immunoassay (ELISA) to detect Sudan Red(SUD) in the sample such as tomato sauce and chili sauce. The kit is composed of Microtiter Plate coated with coupled antigens, HRP conjugate, antibodies, standards and other supporting reagents. During the detection, with adding standards or samples, the SUD in the samples will compete with the coupled antigens to combine with anti-SUD antibodies. After adding HRP conjugate, take coloration with TMB substrates. Absorbance value of the samples is a negative correlation with SUD content. Lastly, by comparing the obtained absorbance values with the standard curve, we can calculate the SUD content in the sample.

Storage conditions

- The kit shall be stored at 2-8 °C. Avoid freezing.
- Shelf life: 12 months. The date of manufacture is presented in the label of the box.

Technique Data

- Kit Sensitivity: 0.3ppb (ng/mL)
- Reactive Mode: 25°C, 30min~30min~15min
- Detection Limits:

| Sample | Detection Limits |
|---|------------------|
| Tomato juice, tomato sauce, chili sauce | 12ppb |
| Chili powder, feed | 120ppb |
| Poultry egg | 30ppb |

- Cross-reaction Rate:

Sudan Red.....100%
Para Red.....123%
Rhodamine.....8%

- Sample Recovery Rate:

| Sample | Recovery Rate |
|---|---------------|
| Tomato juice, tomato sauce, chili sauce | 80±15% |
| Chili powder, feed | 95±15% |
| Poultry egg | 80±15% |

Composition of the Kit

| Reagent | Specification |
|--|------------------|
| Microtiter Plate | 8wells× 12strips |
| High Standard (black cap): 1.0ppm (The Solution is volatile and needs to be sealed) | 1×1.0mL |
| Empty bottles(black cap; You need to prepare solutions before use) | 6 Vials |
| Antibody Solution (blue cap) | 1×5.5mL |
| HRP Conjugate (red cap) | 1×11mL |
| Substrate Reagent A (white cap) | 1×6mL |
| Substrate Reagent B (black cap) | 1×6mL |
| Stop Solution (yellow cap) | 1×6mL |
| Concentrated Wash Buffer (20×) (white cap) | 1×40mL |
| Instruction | 1 |
| Adhesive Membrane | 1 |
| Sealed bag | 1 |

Materials Required but Not Supplied

- **Equipment:** microplate reader, printer, grinder (for homogenizing solid samples), nitrogen evaporator, vortex mixer (**for shake and mix**), centrifuge, graduated transfer pipette, and balance with a division value of 0.01 g, constant temperature device;
- **Micropipette:** single-channel (20-200µL, 100-1000µL), and multi-channel 300µL;
- **Reagents:** Methanol.

Experimental preparation

Please note that the labware must be clean. Use disposable pipette tips to avoid contamination of interference results.

◆ Solution preparation:

Solution 1: 10% Methanol

Mix 10mL of methanol with 90mL of deionized water, and seal tightly for storage.

Solution 2: Working wash buffer

Dilute the concentrated wash buffer (20×) by a factor of 20 (Concentrated wash buffer/Deionized water= 1: 19).

◆ Sample pretreatment steps:

1. Tomato juice, tomato sauce, chili sauce treatment.

- 1) Weigh $2\text{g} \pm 0.05\text{g}$ of homogenized sample into a centrifuge tube, add 10mL methanol, shake for 5 minutes, and centrifuge at room temperature at 4000 r/min for 10 minutes.
- 2) Mix 100 μL of the supernatant with 700 μL deionized water.
- 3) Take 50 μL for analysis.

Dilution times of the sample:40 Detection limits: 12ppb

2.Chili powder, feed treatment.

- 1) Weigh $1\text{g} \pm 0.05\text{g}$ of sample into a centrifuge tube, add 10mL methanol, shake for 5 minutes, and centrifuge at room temperature at 4000 r/min for 10 minutes.
- 2) Mix 20 μL of the supernatant with 780 μL of **10% methanol (Solution 1)**.
- 3) Take 50 μL for analysis.

Dilution times of the sample:400 Detection limits: 120ppb

3. Poultry egg treatment.

- 1) Use a homogenizer to homogenize the poultry egg sample at low speed (for cooked eggs, sample the yolk; for raw eggs, sample the whole egg).
- 2) Weigh $1\text{g} \pm 0.05\text{g}$ of the homogenized egg sample into a

centrifuge tube, add 9mL methanol, shake for 5 minutes (shake vigorously to break up clumps and mix thoroughly), and centrifuge at room temperature at 4000 r/min for 10 minutes.

3) Mix 100 μ L of the supernatant with 900 μ L deionized water

4) Take 50 μ L for analysis.

Dilution times of the sample:100 Detection limits: 30ppb

ELISA procedure

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

Before starting the experiment, it is necessary to prepare the standards (Prepare the low-concentration standard when it is to be used as it is unstable; The prepared standard is stable at 4°C for a month).

Add 3mL of **10% Methanol (Solution 1)** to the vial labeled as 0ppb. Add 2mL of **Solution 1** to the vials labeled as 0.3ppb, 0.9ppb, 2.7ppb, and 8.1ppb, respectively. Add 2.93mL of **Solution 1** to the vial labeled as 24.3ppb.

Standard 6: Pipette 73 μ L of the 1.0ppm **high standard** to the vial labeled as 24.3ppb (containing 2.93mL of **Solution 1**). Close the vial tightly, mix thoroughly, and the concentration will be **24.3ppb**.

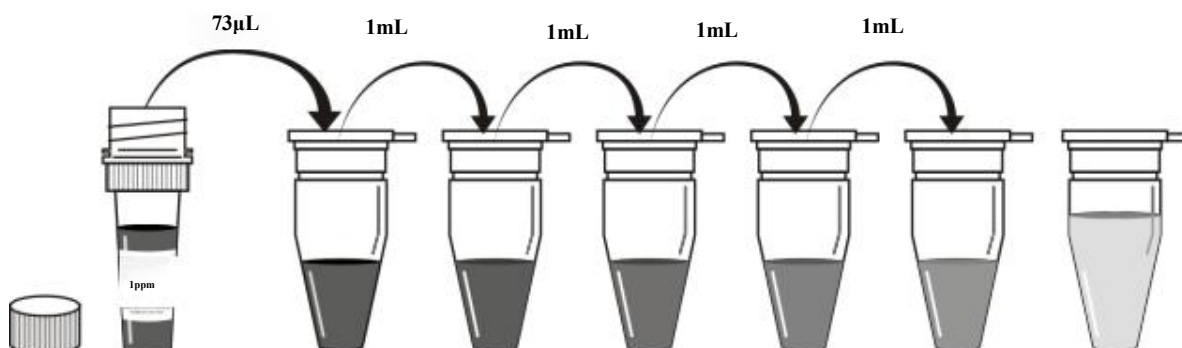
Standard 5: Pipette 1mL of **standard 6** to the vial labeled as 8.1ppb (containing 2mL of **Solution 1**). Close the vial tightly, mix thoroughly, and the concentration will be **8.1ppb**.

Standard 4: Pipette 1mL of **standard 5** to the vial labeled as 2.7ppb (containing 2mL of **Solution 1**). Close the vial tightly, mix thoroughly, and the concentration will be **2.7ppb**.

Standard 3: Pipette 1mL of **standard 4** to the vial labeled as 0.9ppb (containing 2mL of **Solution 1**). Close the vial tightly, mix thoroughly, and the concentration will be **0.9ppb**.

Standard 2: Pipette 1mL of **standard 3** to the vial labeled as 0.3ppb (containing 2mL of **Solution 1**). Close the vial tightly, mix thoroughly, and the concentration will be **0.3ppb**.

Standard 1: The **Solution 1** can be directly used, and the concentration is **0 ppb**.



| Tube | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|------|------|---------|--------|--------|--------|--------|------|
| Con | 1ppm | 24.3ppb | 8.1ppb | 2.7ppb | 0.9ppb | 0.3ppb | 0ppb |

Step 1: Number: Number the samples and standard corresponding to wells in order, make 2-well parallel trials for each sample and standard, and record the locations of sample wells and standard wells.

Step 2: Sample Incubation: Add 50µL of **standard or sample** into each numbered well, then add 50µL of **antibody solution** per well. Finally, cover the Microtiter Plate with the adhesive membrane, shake gently by hand (or use a microplate shaker) for 5s and incubate for 30 min at 25°C.

Step 3: Washing: Uncover the adhesive membrane carefully, remove the liquid, pipette 350µL of **Working Wash Buffer (Solution 2)** to every well, let stand for 30 seconds then drain, repeat 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after patting dry can be punctured with a clean pipette tip).

Step 4: Enzyme Incubation: Add 100µL of **HRP conjugate** into each well. Then cover the Microtiter Plate with the adhesive membrane, shake gently by hand (or use a microplate shaker) for 5s and incubate for 30 min at 25°C.

Step 5: Washing: Same as step 3.

Step 6: Color: Add 50µL of **Substrate Reagent A** to each well. Then add 50µL of **Substrate Reagent B** per well. Shake gently by hand (or use a microplate shaker) for 5s, and allow to react for 15min at 25°C in the dark. (The reaction can be extended

appropriately if the blue color is too pale.)

Step 7: Stop the reaction: Pipette 50µL of **Stop Solution** to each well, and shake gently by hand (or use a microplate shaker). The reaction would be stopped.

Step 8: Calculate: Determine the Optical Density (OD value; absorbance value) at 450nm (Reference wavelength 630nm) with a microplate reader. Finish this step within 10min after stop the reaction.

Interpretation of result

◆ Calculate the percentage of absorbance value

$$\text{Percentage of absorbance value(\%)} = \frac{A}{A_0} \times 100\%$$

A—the average OD value of the sample or the standard;

A₀—the average OD value of the 0ppb standard (double wells).

It is used to calculate the percentage absorbance of a standard or sample.

◆ Draw the standard curve and calculate

- Take absorbance percentage(A/A₀) of standards as Y-axis and the corresponding log of standards concentration (ppb) as X-axis.
- Draw the standard semi-log curves with X-axis and Y-axis.
- Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve. **Last, the resulting concentration values multiplied by the corresponding dilution times is the actual concentration of SUD of samples.**

If professional analysis software of the kit is used for calculation, it is more convenient for accurate and rapid analysis of a large number of samples.

Attention

- Before test, the reagents and samples should be balanced to room temperature (25°C). If below 25°C, it will lead to all the standard OD value on the low side.
- In washing process, dry wells may result in non-linear standard curves and undesirable reproducibility. Therefore, proceed to the next step immediately after washing.

- Please mix the contents within the wells uniformly and wash the plate thoroughly. The reproducibility is largely determined by consistency of washing step.
- During the incubation, cover microplates with adhesive membrane to avoid light.
- Do not use kits that are overdue. Do not mix reagents with those from other lots.
- Substrate Reagent A/B is colorless. If not, please discard.
- If absorbance value of 0ppb is below 0.5 ($A_{450nm} < 0.5$), it means that the reagent may be metamorphic.
- Stop solution is corrosives. Please avoid contacting with skin.
- **As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.**
- **For the mentioned sample, fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.**
- The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS can be used for quantitative confirmation.



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