Catalog Number：AE81052SF

Size: 96 Tests

Instruction manual

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Sudan Red (SUD) ELISA Kit

【*INTENDED USE*】

For the quantitative detection of **Sudan Red (SUD)** concentration in **tomato juice, tomato sauce, chili sauce, chili powder, eggs, feed.**

This package insert must be read in its entirety before using this product.

If You Have Problems

Our expert Technical Support Staff is available to assist you in answering your questions and resolving issues to ensure complete customer satisfaction.

Please Contact Us

Tel: (86)-27-65523378
Fax: (86)-27-65523378
Email: sales@abebio.com
service@abebio.com
Website: www.abebio.com

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

【*REAGENTS AND MATERIALS PROVIDED*】

Reagents	Quantity	Reagents	Quantity
Assay plate (96 Wells)	1	Instruction manual	1
Standard (liquid)	1 x 1 mL	Antibody	1 x 6 mL
HRP-Conjugate	1 x 11 mL	Wash Buffer (concentrate 20 x)	1 x 20 mL
Substrate A	1 x 6 mL	Substrate B	1 x 6 mL
Stop Solution	1 x 6 mL	Adhesive Films	4

【*MATERIALS REQUIRED BUT NOT SUPPLIED*】

※ Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at the dual-wavelength 450/630 nm.

※ Equipments: microplate reader, printer, homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipets, balance (a reciprocal sensibility of 0.01 g).

※ Micropipettors: single-channel 20-200 µL, 100-1000 µL, and multi-channel 250 µL.

※ Reagents: Methanol (CH3OH), deionized water.

【*STORAGE*】

<table>
<tr><td>Unopened kit</td><td colspan="2">Store at 2 - 8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="8">Opened/ Reconstituted Reagents</td><td>Coated assay plate</td><td>May be stored for up to 1 month at 2 - 8°C. Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal, and avoid the damp.</td></tr>
<tr><td>Standard</td><td rowspan="7">May be stored for up to 1 month at 2 - 8°C.</td></tr>
<tr><td>Antibody</td></tr>
<tr><td>HRP-Conjugate</td></tr>
<tr><td>Wash Buffer</td></tr>
<tr><td>Substrate A</td></tr>
<tr><td>Substrate B</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

【*INTRODUCTION*】

Sudan red is an organic compound, typically classified as an azo dye. It is an intensely orange-red solid that is added to colourise waxes, oils, petrol, solvents, and polishes. Sudan I has also been adopted for colouring various foodstuffs, especially curry powder and chili powder, although the use of Sudan I in foods is now banned in many countries, because Sudan I, Sudan III, and Sudan IV have been classified as category 3 carcinogens (not classifiable as to its carcinogenicity to humans) by the International Agency for Research on Cancer. Sudan I is still used in some orange-coloured smoke formulations and as a colouring for cotton refuse used in chemistry experiments.

【*PRINCIPLE OF THE ASSAY*】

This assay is based on the competitive enzyme immunoassay for the detection of SUD in the sample. The coupling antigens are pre-coated on the micro-well stripes. The SUD in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-SUD antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the SUD in it. This value is compared to the standard curve and the SUD concentration is subsequently obtained.

【*LIMITATIONS OF THE PROCEDURE*】

※ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
※ The kit should not be used beyond the expiration date on the kit label.
※ Do not mix or substitute reagents with those from other lots or sources.
※ It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
※ If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
※ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
※ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the ELISA Kit, the possibility of interference cannot be excluded.

【*DETECTION RANGE*】

0.3 ppb - 24.3 ppb. The standard curve concentrations used for the ELISA's were 24.3 ppb, 8.1 ppb, 2.7 ppb, 0.9 ppb, 0.3 ppb, 0 ppb.

Tube	S5	S4	S3	S2	S1	S0
ppb	24.3	8.1	2.7	0.9	0.3	0

【*TECHNICAL SPECIFICATIONS*】

Sensitivity: 0.3 ppb

Detection limit:

Tomato juice, Tomato sauce, Chili sauce	12 ppb
Chili powder, Feed	120 ppb
Eggs (chicken egg, duck egg, goose egg)	30 ppb

Cross-reaction rate:

SUD	100%
Pigment red	123%
Rhodamine	8%

Recovery rate:

Tomato juice, Tomato sauce, Chili sauce	80±15%
Chili powder, Feed	95±15%
Eggs (chicken egg, duck egg, goose egg)	80±15%

【*PRECISION*】

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.
Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.
CV(%) = SD/meanX100
Intra-Assay: CV<8%

Inter-Assay: CV<12%

【*STABILITY*】

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% within the expiration date under appropriate storage condition.

The loss rate was determined by accelerated thermal degradation test. Keep the kit at 37°C for 4 and 7 days, and compare O.D.values of the kit kept at 37°C with that of at recommended temperature. (referring from China Biological Products Standard, which was calculated by the Arrhenius equation. For ELISA kit, 4 days storage at 37°C can be considered as 6 months at 2-8°C, which means 7 days at 37°C equaling 12 months at 2-8°C).

Note:

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

【*SAMPLE PRE-TREATMENT*】

Instructions (The following points must be dealt with before the pre-treatment of any kind of sample)**:**

1. Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents;
2. Before the experiment, each experimental utensil must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution preparation before sample pre-treatment:

1. **10% methanol solution**: dissolve 10 mL Methanol in deionized water to 100 mL.
2. **Washing buffer (1 x)**: dilute 20 mL of the concentrated washing buffer (20×concentrated) with the distilled or deionized water at 1:19 to 400 mL (or just to the required volume) for use.

3. The standard working solution needs to be prepared before the experiment. Low concentration standard is unstable and needs to be used right after prepared. Add 2 mL of the 10% methanol solution to 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb vial respectively and add 2.93 mL of the 10% methanol solution to 24.3 ppb vial.

   Standard solution 5: add 73 μL of 1.0 ppm high concentration standard solution to 24.3 ppb vial containing 2.93 mL 10% methanol solution, tightly capped and completely mixed, the concentration will be 24.3 ppb.

   Standard solution 4: add 1 mL of **Standard solution 5** to 8.1 ppb vial containing 2 mL of the 10% methanol solution, tightly capped and completely mixed, the concentration will be 8.1 ppb.

   Standard solution 3: add 1 mL of **Standard solution 4** to 2.7 ppb vial containing 2 mL of the 10% methanol solution, tightly capped and completely mixed, the concentration will be 2.7 ppb.

   Standard solution 2: add 1 mL of **Standard solution 3** to 0.9 ppb vial containing 2 mL of the 10% methanol solution, tightly capped and completely mixed, the concentration will be 0.9 ppb.

   Standard solution 1: add 1 mL of **Standard solution 2** to 0.3 ppb vial containing 2 mL of the 10% methanol solution, tightly capped and completely mixed, the concentration will be 0.3 ppb.

   Standard solution 0: add 1 mL of the 10% methanol solution to 0 ppb vial, the concentration will be 0 ppb.

Samples preparation:

Tomato juice, tomato sauce, chili sauce;

1. Homogenize the sample.
2. Weigh 1± 0.05 g homogenized samples into centrifuge tube.
3. Dissolve in 10 mL of the methanol, shake strongly for 5 minutes.
4. Centrifuge at above 4000 r/min at room temperature (20-25°C) for 10 minutes.
5. Take 100 μL of the supernatant into a new centrifuge tube, add 700 μL of the deionized water, mix well.
6. Take 50 μL for further analysis.

Fold of dilution of sample: 40

Chili powder, feed;

1. Homogenize the sample.
2. Weigh 1± 0.05 g homogenized samples into centrifuge tube.
3. Dissolve in 10 mL of the methanol, shake strongly for 5 minutes.
4. Centrifuge at above 4000 r/min at room temperature (20-25°C) for 10 minutes.
5. Take 20 μL of the supernatant into a new centrifuge tube, add 780 μL of the 10% methanol solution, mix well.
6. Take 50 μL for further analysis.

Fold of dilution of sample: 400

Eggs (chicken egg, duck egg, goose egg);

1. Homogenize the egg sample (sample yolk from cooked egg; sample whole egg liquid from raw egg) with a homogenizer at low speed.
2. Weigh 1± 0.05 g homogenized samples into centrifuge tube.
3. Dissolve in 9 mL of the methanol, shake strongly for 5 minutes (vibrate it violently to disperse those clouds of samples and mix them well).
4. Centrifuge at above 4000 r/min at 15°C for 10 minutes.
7. Take 100 μL of the supernatant into a new centrifuge tube, add 900 μL of the deionized water, mix well.
5. Take 50 μL for further analysis.

Fold of dilution of sample: 100

【*SAMPLE STORAGE*】

1. Untreated samples are stored at frozen environment.
2. Prepared sample can be stable at 2-8°C for 1 week.

【*TABLE*】

Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 5 (24.3 ppb)	Standard 5 (24.3 ppb)	Sample 3	Sample 3
B	Standard 4	Standard 4	Sample 4	Sample 4

	(8.1 ppb)	(8.1 ppb)		
C	Standard 3 (2.7 ppb)	Standard 3 (2.7 ppb)	Sample 5	Sample 5
D	Standard 2 (0.9 ppb)	Standard 2 (0.9 ppb)	Sample 6	Sample 6
E	Standard 1 (0.3 ppb)	Standard 1 (0.3 ppb)	Sample 7	Sample 7
F	Standard 0 (0 ppb)	Standard 0 (0 ppb)	Sample 8	Sample 8
G	Sample 1	Sample 1	Sample 9	Sample 9
H	Sample 2	Sample 2	Sample 10	Sample 10

【*ASSAY PROCEDURE*】

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Bring test kit to the room temperature (20-25°C) for at least 30 minutes, note that each reagent must be shaken evenly before use; put the required micro-well strips into plate frames. Re-sealed the unused microplate, stored at 2-8°C, not frozen.
2. Add 50 μL of the sample or standard solution into separate wells; and add 50 μL of the antibody working solution into each well, mix gently by shaking the plate manully. Seal the microplate with the cover membrane, and incubate at 25°C for 30 minutes.
3. Pour the liquid, wash the microplate with the washing buffer at 250 μL/well for 5 times. Each time soak the well with the washing buffer for 15-30s, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
4. Add 100 μL of HRP-Conjugate to each well. Cover the microtiter plate with the adhesive films. Incubate for 30 minutes at 25°C.
5. Pour the liquid, wash the microplate with the washing buffer at 250 μL/well for 5 times. Each time soak the well with the washing buffer for 15-30s, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).

6. Add 50 μL of Substrate A and 50 μL of Substrate B to each well, mix well. Incubate for 15 minutes at 25°C. Keeping the plate away from drafts and other temperature fluctuations in the dark. Avoid placing the plate in direct light.
7. Add 50 μL of Stop Solution to each well. when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 630 nm. Subtract readings at 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

【*CALCULATION OF RESULTS*】

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the SUD concentration in the sample.

1) Qualitative determination

The concentration range (ppb) of SUD can be obtained from comparing the average OD value of the sample with that of the standard solution.

2) Quantitative determination

The mean values of the absorbance values obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is

$$\text{Percentage of absorbance value} = \frac{B}{B0} \times 100\%$$

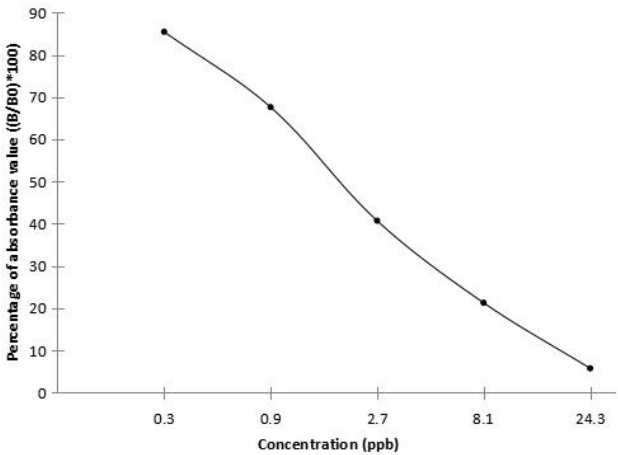
B—the average (double wells) OD value of the sample or the standard solution
B0—the average OD value of the 0 ppb standard solution

Draw the standard curve with the absorption percentages of standard solutions and the semilogarithm values of SUD standard solutions (ppb) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the dilution fold, finally obtaining SUD concentration in the sample.

Using the professional software of this kit will be more convenient for accurate and rapid analysis of a large amount of samples.

【*TYPICAL DATA*】

For convenience in result calculation, absorbance as abscissa and standard concentrations can be used as ordinate. The standard curve provided in the manual is only for reference, experimenters should draw the standard curve based on data of themselves.



Precautions: This kit contains trace amount of proclin® 300 which is toxic and direct touch of reagents by mouth, eyes, and skin should be avoided. If get touched or get into the mouth, please rinse with plenty of water. The Stop Buffer is a corrosive sulfuric acid solution, please take care for safety.

【*IMPORTANT NOTE*】

1. The instruction manual also suits for the kit of 48T, but all reagents of 48T kit are reduced by half.
2. There may be some foggy substance in the wells when the plate is opened at the first time. It will not effect on the final assay results. Do not remove microtiter plate from the storage bag unless needed.
3. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
4. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as far as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
5. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
6. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
7. Controlling of reaction time: Observe the change of color after adding Substrate Solution (e.g. observation once every 10 minutes), Substrate Solution should change from colorless or light blue to gradations of blue. If

the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

8. Substrate Solution is easily contaminated. Substrate Solution should remain colorless or light blue until added to the plate. Please protect it from light.
9. Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
10. Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism.
11. Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbance measurement.
12. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.
13. Limited by the current condition and scientific technology, we can't completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit.
14. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
15. Kits from different batches may be a little different in detection range, sensitivity and color developing time.
16. Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments.

Intra-assay variance among kits from different batches might arise from above factors, too.

17. Kits from different manufacturers with the same item might produce different results, since we haven't compared our products with other manufacturers.
18. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
19. Valid period: six months.