Importance of Sudan Red Determination

Sudan dyes are inexpensive fat-soluble azo compounds, used mainly as colorants in the manufacturing of oils, wax products, lacquers and inks since their bright and vivant colors can improve the luster of commercial products. Sudan I is classified as a carcinogen by the International Agency for Research in Cancer (IARC). Sudan II, the dimethyl derivative of Sudan I has been shown to cause bladder carcinomas in mice at a high frequency. Due to their potential carcinogenicity, many countries have banned the use of Sudan dyes (I - IV) in products for human consumption. Similar to Sudan I, Para Red has also been shown to be carcinogen.

Sudan dyes are often used illegally to enhance and/or maintain the appearance of food products, such bell peppers, curry, chili powders, etc. Contamination of meat products have also occurred. Therefore, control of Sudan Red dyes in food products is crucial to insure food safety.

The Sudan Red ELISA allows the determination of 42 samples in duplicate determination. The test can be performed in approximately 1 hour.

Performance Data

Test sensitivity: The detection limit for Sudan Red is 0.11ng/mL (mean of 6 blank determinations minus 3 standard deviations). The middle of the test (50% B/B_0) is at approximately 1.25 ng/mL. Determinations closer to the middle of the calibration curve give the most accurate results.

Test reproducibility: Coefficients of variation (CVs) for standards: <10%; CVs for samples: <15%.

Specificity: The cross-reactivity of the Abraxis Sudan Red Kit for various Sudan dyes and analogues are as follows:

Compound	90 % B/Bo (ppb)	50 % B/Bo (ppb)
Sudan I	0.1	1.3
Sudan II	0.70	4.6
Sudan III	0.45	9.7
Sudan IV	3.19	87
Sudan Red G	0.05	1.5
Tropaeolin	0.05	1.3
Para Red	0.2	6.6
Sunset Yellow	>100	>100

Recoveries: Recoveries of Sudan Red from spiked matrices and after recommended dilution were between 84-113%.

General Limited Warranty: Abraxis LLC warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose**

For ordering or technical assistance contact:

Abraxis LLC
54 Steamwhistle Drive
Warminster, PA 18974
Tel.: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: *WWW.abraxiskits.com*

R052809

Sudan Red ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Sudan Red Dyes in Contaminated Samples

Product No. 515106

1. General Description

The Sudan Red ELISA is an immunoassay for the quantitative and sensitive screening of Sudan Red and analogs. This test is suitable for the quantitative and/or qualitative screening of Sudan Red in food samples such as tomato juice, tomato sauce, chili sauce, chili powder, etc. (please refer to the appropriate technical bulletins for extraction/dilution procedures). Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions of the test kit contain small amounts of Sudan Red. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The Sudan Red ELISA should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box.

4. Test Principle

The test is a direct competitive ELISA and it is based on the recognition of Sudan Red by antibodies. Sudan Red when present in calibrators or samples and a Sudan Red-enzyme (HRP) analogue compete for the binding sites of rabbit-anti-Sudan Red antibodies in solution. The Sudan Red antibodies are bound by a second antibody (anti-rabbit) immobilized in the plate. After a washing step and addition of a substrate solution, a color signal (blue) is generated. Following a 20 minute incubation, the reaction is stopped by the addition of diluted acid and the amount of color (yellow) in each well is read using an ELISA reader. The color of the unknown samples is compared to the color of the calibrators and the Sudan Red concentration of the samples is derived. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Sudan Red ELISA, Possible Test Interference

Testing of food samples such as tomato juice, tomato sauce, chili sauce, and chili powder have shown that a dilution (in dilution buffer) is necessary to eliminate matrix effects. Recommendation for dilution of the listed food samples can be found in Section H. Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects can't be completely excluded. Other food sources might require different dilution scheme and should be validated.

Mistakes in handling the test also can cause errors. Possible sources for such errors can be: inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme outside temperatures (lower than 10 ^{0}C or higher than 30 ^{0}C) during the test performance.

The Abraxis Sudan Red ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.), samples requiring some regulatory action should be confirmed by an alternative method.

A. Reagents and Materials Provided

1. Microtiter plate (12 X 8 strips) coated with anti-rabbit antibody, in an resealable aluminum pouch
2. Calibrators/Standards (6): 0, 0.25, 0.50, 1.0, 2.5, 5.0 ng/mL (ppb) of Sudan Red I, 1 mL each
3. Sudan Red-HRP Conjugate, 6 mL
4. Anti-Sudan Red Antibody Solution, 6 mL
5. Wash Solution (5X) Concentrate, 100 mL. Must be diluted 1:5 with deionized water before use
6. Substrate (Color) Solution (TMB), 12 mL
7. Stop Solution, 6 mL (Handle with care)
8. Sample Diluent, 30 mL

B. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the enzyme conjugate, antibody solution, substrate solution and the stop solution in order to equalize the incubation periods on the entire microtiter plate. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the aluminum pouch. The remaining strips are stored in the aluminum pouch and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions, enzyme conjugate, antibody solution, substrate and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the wash buffer at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water.
5. The stop solution must be handled with care as it contains diluted H_2SO_4.

C. Assay Procedure

1. Add 50 µL of the **standard solutions** or the **sample extracts (Section H)** into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50 µL of **enzyme conjugate** solution to the individual wells successively using a multi- channel pipette or a stepping pipette.
3. Add 50 µL of **antibody** solution to the individual wells successively using a multi- channel pipette or a stepping pipette.
4. Cover the wells with parafilm or tape and mix contents by moving the strip holder in a rapid circular motion on the bench top for about thirty (30) seconds. Incubate the strips for 45 minutes at room temperature.
5. After incubation, remove the covering and vigorously shake the contents of the wells into a sink or suitable waste container.
6. Wash the strips four (4) times using the diluted (1X) **washing buffer solution**. Please use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
7. Add 100 µL of **substrate (color) solution** to the wells. Incubate the strips for 20 min at room temperature. Protect the strips from direct sunlight.
8. Add 50 µL of **stop solution** to the wells in the same sequence as for the substrate solution.
9. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of stopping solution.

D. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as Logit/Log or 4-Parameter (preferred). For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding Sudan Red concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb or ng/mL of Sudan Red by interpolation using the standard curve. Results can also be obtained by using a spreadsheet macro available from Abraxis upon request.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing a lower concentrations of Sudan Red compared to standard 1 (0.25 ng/mL or ppb) must be reported as containing < 0.25 ppb Sudan Red. Samples showing a higher concentration than standard 5 (5 ng/mL) must be diluted further to obtain accurate results.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrators. Sample containing less color than a calibrator will have a concentration of Sudan Red greater than the concentration of the calibrator. Samples containing more color than a calibrator will have a concentration less than the concentration of the calibrator.

E. Additional Materials (not delivered with the test kit)

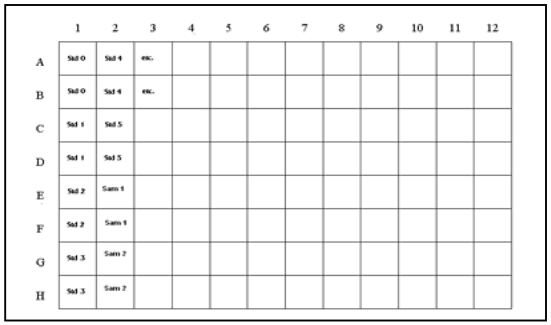
1. Micro-pipettes with disposable plastic tips (50-200 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (50-250 µL)
3. Microtiter plate washer (optional)
4. Microtiter plate reader (wave length 450 nm)
5. Shaker for microtiter plates (optional)
6. Deionized or distilled water
7. Methanol
8. Paper towels or equivalent absorbent material
9. Timer

F. Working Scheme

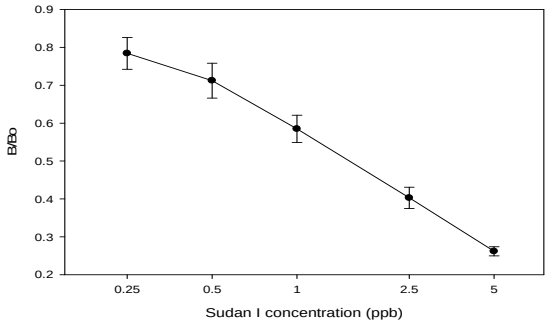
The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 5: Standards
(0; 0.25; 0.50;1.0; 2.5; 5.0 ppb)

Sam1, Sam2, etc.: Samples



G. Standard Curve (These values are used for demonstration purposes; do not use these values for your determinations)



H. Food Sample Extraction

1A- Tomato Juice- To a glass vial, add 1 gm of matrix and 5 mL of methanol
1B- Tomato Sauce- To a glass vial, add 1 gm of matrix and 5 mL of methanol
1C- Chili Sauce- To a glass vial, add 2.5 gm of matrix and 25 mL of methanol
1D- Chili Powder- To a glass vial, add 1 gm of matrix and 10 mL of methanol
2. Vortex or shake for 1-2 minutes. Allow to settle until a visible top clear layer is seen.
3. Dilute samples as follows prior to running in the assay:
- Tomato juice, tomato sauce and chili sauce samples should be diluted 1:25 in sample dilution buffer (50 uL of extract + 1.2 mL sample dilution buffer)
- Chili powder samples should be diluted 1:250 in sample dilution buffer (50 uL of extract + 12.5 mL sample dilution buffer)
4. Highly contaminated samples (samples outside the standard curve range) should be diluted in order to get the value in the middle of the curve and re-analyzed.
5. To obtain the final concentration of the sample, the concentration obtained in the assay **must** be multiplied by the dilution factor.