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Sudan ELISA Kit

10/19

(Catalog # E4769-100; 96 assays, Storage at 4°C)

I. Introduction:

II. Sudan dyes are synthetic chemical dyes of similar chemical structure. They are aromatic compounds containing azo group (- N=N -). Sudan dyes are widely used in commercial manufacturing to impart a rich red, red-orange or yellow-orange color to plastics and textiles. They can be generally applied for coloring substances such as hydrocarbon solvents, oils, fats, waxes and plastics. Although Sudan dyes have been reported as contact allergens and sensitizers, the greatest concern has been on their possible carcinogenicity. BioVision's Sudan ELISA Kit is based on Competitive ELISA method. The microtiter plate provided in the kit has been pre-coated with coupled antigen. During the reaction, Sudan dye in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-Sudan antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and TMB substrate is added for color development. There is a negative correlation between the OD value of samples and the concentration of Sudan. The concentration of Sudan in the samples can be calculated by comparing the OD of the samples to the standard curve.

III. Applications:

In vitro, quantitative determination of Sudan
Detection Range: Tomato juice, Ketchup, Chilli sauce - 12ppb, Chilli powder, Feed -120ppb,
Eggs (Chicken egg, Duck egg, Goose egg) - 30ppb
Sensitivity: 0.3 ppb (ng/mL)

Sample recovery rate: Tomato juice, Ketchup, Chilli sauce - 80%±15%, Chilli powder, Feed - 95%±15%,

Eggs(Chicken egg, Duck egg, Goose egg) - 80%±15%

IV. Sample Type:

Tomato juice, Ketchup, Chilli sauce, Paprika, Feed, Eggs

V. Kit Contents:

Components	E4769-100	Part Number
Micro ELISA Plate	96 wells	E4769-100-1
High concreted Standard (1.0 ppm)	1 ml	E4769-100-2
HRP Conjugate	11 ml	E4769-100-3
Antibody Working Solution	5.5 ml	E4769-100-4
Substrate Reagent A	6 ml	E4769-100-5
Substrate Reagent B	6 ml	E4769-100-6
Stop Solution	6 ml	E4769-100-7
Wash Buffer (20X)	40 ml	E4769-100-8
Empty vials	6	E4769-100-9
Plate Sealer	3	E4769-100-10

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Methanol
- Clean eppendorf tubes for preparing standards or sample dilutions

VII. Storage and Handling:

Store at 4ºC**.**

VIII. Reagent and Sample Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Wash Buffer (20X):** Dilute 20X Concentrated Wash Buffer to 1X with deionized water.
- **10% Methanol:** Add10 ml Methanol to 90 ml deionized water, mix well.

- **Standard:** Prepare fresh standards each time. Label the empty bottles provided as 0 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb and 24.3 ppb. Add 3 ml of 10% Methanol into vial label as 0 ppb. Add 2 ml of 10% Methanol into standard vial label as 0.3 ppb, 0.9 ppb, 2.7 ppb, and 8.1 ppb respectively. Add 2.93 ml of 10% Methanol into 24.3 ppb vial. To prepare standards, mix high concentrated standard (1.0 ppb) with methanol as described below:

  Standard 6 (24.3 ppb): Add 73 µl of 1.0 ppm high concentrated standard into 24.3 ppb vial, mix well.
  Standard 5 (8.1 ppb): Add 1 ml of Standard Solution 6 into 8.1 ppb vial, mix well.
  Standard 4 (2.7ppb): Add 1 ml of Standard Solution 5 into 2.7ppb vial, mix well.
  Standard 3 (0.9 ppb): Add 1 mL of Standard Solution 4 into 0.9 ppb vial, mix well.
  Standard 2 (0.3 ppb): Take 1 mL of Standard Solution 3 into 0.3 ppb vial, mix well.
  Standard 1 (0 ppb): Use 10% Methanol only.

IX. Sample Preparation:

Sample pretreatment:

- **Pretreatment of Tomato juice/Ketchup/chili sauce**
  Weigh 2 ± 0.05 g of homogenate sample into centrifuge tube, add 10 ml Methanol, shake for 5 min, centrifuge at 4000 rpm at room temperature for 10 min. Take 100 µl of supernatant to mix with 700 µl of deionized water. Take 50 µl of the mixture to analyze.
  Note: Sample dilution factor: 40, minimum detection limit: 12 ppb

- **Pretreatment of chilli powder/Feed:**
  Weigh 1 ± 0.05 g of sample into centrifuge tube; add 10 mL of Methanol shake for 5 min, centrifuge at 4000 rpm at room temperature for 10 min. Take 20 µl of supernatant to mix with 780 µl of 10% Methanol. Take 50 µl of the mixture to analyze.
  Note: Sample dilution factor: 400, minimum detection limit: 120 ppb

- **Pretreatment of eggs:**
  Homogeneous egg samples with Homogenizer at low speed Cooked egg sampling egg yolk, raw egg sampling whole egg. Weigh 1 ± 0.05 g of homogenate eggs sample into centrifuge tube, add 9 mL of Methanol, shake for 5 min oscillate violently to separate a group of samples, mixed fully centrifuge at 4000 rpm at 15 for 10 min. Take 100 µl of supernatant; add 900 µL of deionized water, mixed fully. Take 50 µl of the mixture to analyze.
  Note: Sample dilution 100, minimum detection limit: 30 ppb

X. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Add 50 µl of each **standard or samples** into appropriate wells.
2. Immediately add 50 µl of **Antibody working solution** to each well. Cover the plate with the sealer provided in the kit. Gently mix and incubate for 30 min. at 25°C. **Note:** solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and bubble formation as much as possible.
3. Aspirate the solution from each well add 300 µl of **1x wash buffer** to each well. Leave it for 30 sec, aspirate the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 5 times.

   Note: a microplate washer can be used in this step and other wash steps.
4. Add 100 µl of **HRP Conjugate** working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 25°C.
5. Aspirate the solution from each well, repeat the wash process for five times as conducted in step 3.
6. Add 50 µl of **Substrate Reagent A** to each well and then add 50 µl of **Substrate Reagent B.** Cover with a new plate sealer. Incubate for about 15 min at 25°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
7. Add 50 µl of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
8. Read the absorbance in micro plate reader set to 450 nm reference wavelength 630 nm. This step should be performed within 5 min after stop reaction.

XI. Calculation:

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.

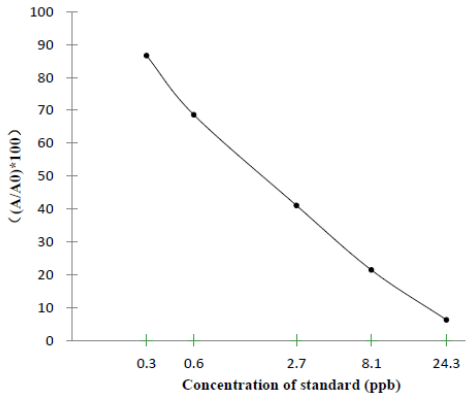
Typical standard curve and data is provided below for reference only. A standard curve must be run with each assay.

Absorbance (%)=A/A_0 ×100%

A: Average absorbance of standard or sample

A_0 : Average absorbance of 0 ppb Standard

Concentration of standard (ppb)	OD-1	OD-2	Average OD
0	2.0200	2.2004	2.1102
0.3	1.8124	1.8436	1.8280
0.6	1.4206	1.4754	1.4480
2.7	0.8614	0.8713	0.8664
8.1	0.4410	0.4631	0.4521
24.3	0.1271	0.1384	0.1328



XII. RELATED PRODUCTS:

- Ciprofloxacin (Cipro) ELISA Kit (E4365)
- Chloramphenicol (CAP) ELISA Kit (K4230)
  Aflatoxin B1 (AFB1) ELISA Kit (K4208)

FOR RESEARCH USE ONLY! Not to be used on humans.