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User's Manual

Sudan ELISA Kit

REF **DEIANJ27**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Sudan ELISA Kit is an immunoassay for the quantitative and sensitive screening of Sudan I, II, III, IV in food samples such as chili sauce and chili powder, etc.

General Description

Sudan is an azo dye. It is added to colourise waxes, oils, petrol, solvents, and polishes. Sudan has also been adopted for colouring various foodstuffs, especially curry powder and chili powder, although the use of Sudan 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.

Principles of Testing

The test is a direct competitive ELISA and it is based on the recognition of Sudan by antibodies. Sudan, when present in calibrators or samples and a Sudan-enzyme (HRP) analogue compete for the binding sites of anti-Sudan antibodies in solution. The Sudan antibodies are bound by a second antibody 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 concentration of the samples is derived. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

Reagents And Materials Provided

1. Microtiter plate (12 X 8 strips) coated with antibody, in an resealable aluminum pouch
2. Calibrators/Standards
3. Sudan-HRP Conjugate
4. Anti-Sudan Antibody Solution
5. Wash Solution
6. Substrate (Color) Solution (TMB)
7. Stop Solution
8. Sample Diluent

Materials Required But Not Supplied

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

Storage

Store the kit at 2 - 8°C.

Specimen Collection And Preparation

1. Chili Sauce-To a glass vial, add 2.5 gm of matrix and 25 mL of methanol

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.
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.

Assay Procedure

1. Add 50 µL of the standard solutions or the sample extracts into the wells of the test strips. 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 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 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.