Importance of Yellow Azo Dyes Determination

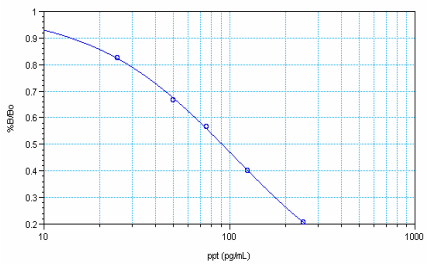
Many foods contain Yellow Azo dyes in varying proportions, due to food sensitivities and intolerance there is a trend to avoid them or substitute them for a non-synthetic or natural substances. It has been reported that Tartrazine causes the most allergic and intolerance reactions all of the Yellow Azo dyes, particularly among asthmatics and those with aspirin intolerance. Symptoms of yellow azo dye sensitivity can occur by either ingestion or cutaneous exposure to a substance containing Yellow Azo dyes. A variety of immunologic responses have been attributed to tartrazine ingestion, including anxiety, migraines, clinical depression, blurred vision, itching, general weakness, heatwaves, feeling of suffocation, purple skin patches, and sleep disturbance. Certain people exposed to Yellow Azo dyes experience symptoms of sensitivity even at extremely low doses, some for periods up to 72 hours after exposure. In children, asthma attacks and hives have been claimed, as well as links to thyroid tumors, chromosomal damage and hyperactivity. Therefore, the monitoring of Yellow Azo dyes in foods is necessary to ascertain that these compounds do not present a danger to consumers with intolerance or sensitivities.

Products that include Yellow Azo dyes include: confectionery, cotton candy, soft drinks, energy drinks, instant puddings, flavored corn chips, cereals, cake mixes, pastries, yellow popcorn, instant or cube soups, sauces, some rice, powdered drink mixes, sport drinks, ice cream, ice pops, candy, jam, jelly, gelatins, marmalade, mustard, horseradish, yogurt, noodles, pickles, fruit cocktails, potato chips, biscuits, vitamins, ant-acids, certain prescriptions drugs, etc. Non food products containing Yellow Azo dyes include: soaps, cosmetics, shampoos and other hair products, moisturizers, crayons, hand sanitizers, nail polish, inks, etc.

The Abraxis Yellow Azo dyes ELISA allows the determination of 41 samples in duplicate determination. Only a few milliliters of sample are required. The test can be performed in less than 2 hours.

Performance Data

Test sensitivity: The limit of detection forYellow Azo dyes calculated as Xn +/- 3SD (n=20) or as 90% B/Bound is equal to 15 pg/mL. The concentration of residue necessary to cause 50% inhibition (50% B/B_0) is approximately 90 pg/mL. Determinations closer to the middle of the calibration range of the test yield the most accurate results.



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

Selectivity: This ELISA recognizes several Yellow Azo dyes with varying degrees:

Cross-reactivities:

		IC-50 (pg/mL)
Tartrazine (FD & C Yellow 5)	100%	98
Yellow 2G	2.0%	4,700
Acid Yellow	1.56%	6,000
Tropaeolin	0.025%	380,000
Sunset Yellow	0.005%	1,900,000
Orange 11	0.0014%	6,700,000
Methyl Orange	<0.0009	>10,000,000
4'amino Azobenzene	no reactivity up to 2,000,000 pg/mL	

Samples: Numerous solid and liquid samples. See Preparation of Samples section.

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ABRAXIS

Yellow Azo Dyes ELISA, Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Yellow Azo Dyes in a Variety of Sample Matrixes

Product No. 522515

1. General Description

The Abraxis Yellow Azo dyes ELISA is an immunoassay for the detection of Yello Azo dyes (Tartrazine and related dyes). This test is suitable for the quantitative and/or qualitative detection of Yellow Azo dyes in contaminated samples. Positive samples should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in this test kit contain small amounts of Tartrazine. 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 Yellow Azo dyes ELISA should to 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 based on the recognition of Tartrazine and related compounds by specific antibodies. Yellow Azo dyes, when present in a sample, and a Tartrazine-enzyme conjugate compete for the binding sites of rabbit anti-Yellow Azo dyes antibodies in solution. The Yellow Azo dyes antibodies are then bound by a second antibody (goat anti-rabbit) immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of Yellow Azo dyes present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Yellow Azo dyes ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects can not be completely excluded. Mistakes in handling the test can also cause errors. Possible sources for such errors can be:

Inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

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

Working Instructions

A. Materials Provided

1. Microtiter plate coated with a second antibody (goat anti-rabbit).
2. Tartrazine Standards (6): 0, 25, 50, 75, 125, 250 pg/mL.
3. Antibody Solution (rabbit anti-Yellow Azo dyes), 6 mL.
4. Yellow Azo dyes -HRP Conjugate, 6 mL.
5. Sample Diluent, 25 mL. Use to dilute samples.
6. Wash Solution (5X) Concentrate, 100 mL.
7. Color (Substrate) Solution (TMB), 16 mL.
8. Stop Solution, 12 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 conjugate, antibody, substrate and stop solutions in order to equalize the incubations periods of the solutions on the entire microtiter plate. Please use only 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 foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions, enzyme conjugate, antibody, substrate and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the wash buffer concentrate 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 should be handled with care as it contains diluted H_2SO_4.

C. Assay Procedure

1. Add 50 µL of the standard solutions or samples (sample extracts) 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. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Be careful not to spill contents.
4. Incubate the strips for 60 minutes at room temperature.
5. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Wash the strips three times using the 1X washing buffer solution. 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.
6. Add 150 µL of substrate (color) solution to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Incubate the strips for 20 minutes at room temperature. Protect the strips from direct sunlight.
7. Add 100 µL of stop solution to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

D. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log. For 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 β-agonist concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb of a β-agonist by interpolation using the standard curve. Samples showing lower concentrations of β-agonist compared to Standard 1 (25 pg/mL) are considered as negative. Samples showing a higher concentration than Standard 5 (250 pg/mL) must be diluted further to obtain accurate results.

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

1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
2. Multi-channel pipette (10-250 µL) or stepper pipette with plastic tips (10-250 µL)
3. Microtiter plate washer (optional)
4. Microtiter plate reader (wave length 450 nm)
5. Shaker for microtiter plates (optional)
6. Timer
7. Tape or Parafilm
8. Methanol, Phosphate monobasic and dibasic salts, sodium chloride

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; 25; 50; 75; 125; 250 ppb

Sam1, Sam2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	etc.									
B	Std 0	Std 4	etc.									
C	Std 1	Std 5										
D	Std 1	Std 5										
E	Std 2	Sam 1										
F	Std 2	Sam 1										
G	Std 3	Sam 2										
H	Std 3	Sam 2										

G. Preparation of Samples

Sample Extraction: Cereals, Mexican/Spanish Rice, Corn Chips, Cheese Snacks, Macaroni and cheese

1. Weigh 0.5 g of homogenized sample into a 15 mL centrifuge tube.
2. Add 10 mL of 50% Methanol in DI water. Vortex for 30 seconds and then using a tube rotator rotate for 10 minutes to extract..
3. Centrifuge tube 5 minutes at 2,800 X g
4. Transfer a few mLs of supernatant into a clean vial.
5. Dilute supernatant 5X as follows: 200 uL of sample (step 4) + 300 uL of DI water + 500 uL of 40 mM PBS.
6. Dilute sample (step 5) at least 1:1 with Sample Diluent (this will give a 1:200 final dilution). Some samples will need to be diluted further and in some cases up to several million depending on the degree of contamination.

The Yellow Azo dye concentration contained in the samples is then determined by multiplying the ELISA result by the dilution factor of 200 (if used schemed provided) or higher. Highly contaminated samples outside of the calibration range of the assay must be diluted further and re-analyzed. The LOQ in solid samples is 5 ng/gm.

Liquid Sample Preparation: Soda, Sports Drinks, etc.

1. Measure and add 0.5 mL of liquid sample into a 15 mL centrifuge tube.
2. Add 9.5 mL of 50% Methanol in DI water. Vortex for 30 seconds and then using a tube rotator rotate for 10 minutes to extract.
3. Dilute 5X as follows: 200 uL of sample (step 2) + 300 uL of DI water + 500 uL of 40 mM PBS.
4. Dilute sample (step 3) at least 1:1 with Sample Diluent (this will give a 1:200 final dilution). Some samples will need to be diluted further and in some cases up to several million depending on the degree of contamination.

The Yellow Azo dye concentration contained in the samples is then determined by multiplying the ELISA result by the dilution factor of 200 (if used schemed provided) or higher. The LOQ in liquid samples is 5 ng/mL.

For additional extraction procedures for various matrices please contact Abraxis LLC.

Preparation of Solutions:

50% Methanol/DI Water: Dilute 100 mL of Methanol with 100 L of deionized or distilled water.

40mM Phosphate Buffered Saline (PBS), pH 7.4: In 800 mL of distilled or deionized water, dissolve 4.554 g of Sodium Phosphate Dibasic anhydrous, 1.096 g of Sodium Phosphate Monobasic monohydrate, and 36.0 g of Sodium Chloride. Bring to 1 L volume with distilled or deionized water. pH= 7.2-7.4.