

### Importance of Melamine Determination

Melamine is an organic base with the chemical formula  $C_3H_6N_6$ , and the IUPAC name 1,3,5-triazine-2,4,6-triamine. Melamine is a trimer of cyanamide. Like cyanamide, it is 66% nitrogen (by mass) and provides flame retardant properties to resin formulas by releasing nitrogen when burned or charred. Dicyandiamide (or cyanoguanidine), the dimer of cyanamide, is also used as a flame retardant. Melamine is a metabolite of cyromazine, a pesticide. It is formed in the bodies of mammals who have ingested cyromazine. Cyromazine is also converted to melamine in plants.

Melamine is used in combination with formaldehyde to produce melamine resin, a very durable thermosetting plastic, and melamine foam, a polymeric cleaning product. The end products containing melamine include countertops, fabrics, glues and flame retardants. Melamine is one of the major components in Pigment Yellow 150, a colorant in inks and plastics. Melamine is also used to make fertilizers.

Ingestion of melamine may lead to reproductive damage, bladder or kidney stones, which can lead to bladder cancer. A study in 1953 reported that dogs fed 3% melamine for a year had the following changes in their urine: (1) reduced specific gravity, (2) increased output, (3) melamine crystalluria, and (4) protein and occult blood.

The practice of adding "melamine scrap" to animal feed in order to give the appearance of increased protein content is reported to be widespread in various countries. Melamine has also been intentionally added as a binding agent in fish and livestock feed. This practice can potentially contaminate animal products intended for human consumption such as meat and dairy products. Melamine has also been directly added to foods intended for human consumption. Recently, several companies and individuals were implicated in a scandal involving milk and infant formula which had been adulterated with melamine, leading to kidney stones and renal failure, causing four known infant deaths, and sickening nearly 53,000 infants.

The Melamine ELISA allows the determination of 42 samples in duplicate determination. Less than a mL of sample extract is required. The test can be performed in less than 1 hour.

### Performance Data

Test sensitivity:

The detection limit for Melamine is 10 µg/L (mean of 6 blank determinations minus 3 standard deviations). The middle of the test (50% B/B<sub>0</sub>) is at approximately 150 µg/L. 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 Melamine Kit for various Triazines can be expressed as the least detectable dose (LDD), which is estimated at 90% B/B<sub>0</sub>, or as the dose required for a 50% absorbance inhibition (50% B/B<sub>0</sub>).

Compound	LDD (ppb)	50% B/B <sub>0</sub> (ppb)
Melamine	10	150
Ammeline	7	140
Ammelide	400	7,000
Cyanuric Acid	400	>10,000
Atrazine	>10,000	>10,000
Diamino Atrazine	0.1	2

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## Melamine ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Melamine in Contaminated Samples

Product No. 50005B

### 1. General Description

The Melamine ELISA is an immunoassay for the quantitative and sensitive screening of Melamine. This test is suitable for the quantitative and/or qualitative screening of Melamine in various sample matrices (please refer to the appropriate technical bulletins for extraction/dilution procedures). If necessary, samples requiring regulatory action can be confirmed by HPLC, GC/MS, or other conventional methods.

### 2. Safety Instructions

The standard solutions of the test kit contain small amounts of Melamine. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted hydrochloric 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 Melamine 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 based on the recognition of Melamine by antibodies. The calibrators, sample extracts, and Melamine HRP conjugate are pipetted into test wells coated with Melamine antibody to initiate the reaction. During the 30 minute incubation period, Melamine from the sample and Melamine HRP conjugate compete for binding to Melamine antibody. Following this 30 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound Melamine and Melamine HRP conjugate. After washing with the diluted wash solution, a clear substrate is then added to the wells and any bound enzyme conjugate causes the conversion to a blue color. Following a 20 minute incubation, the reaction is stopped and the amount of color 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 Melamine 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 Melamine ELISA, Possible Test Interference

Many 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't be completely excluded. Some matrices such as fatty foods, require a simple sample dilution before analysis to eliminate interferences (refer to appropriate Technical Bulletins). 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 during the test performance.

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