APPLICATION NOTE

High-throughput melamine detection with Abraxis Melamine ELISA Kit and Molecular Devices Absorbance readers

Introduction

The organic base melamine is used to make a number of products, including plastics, flame retardants, pigments, and fertilizers. The practice of adding melamine to animal feed and foods for human consumption in order to increase the apparent protein content has recently been reported. Because melamine can cause serious illness or death, there is increased interest in identifying methods for detecting melamine contamination in a variety of food products.[1]

The Abraxis Melamine enzyme-linked immuno-sorbent assay (ELISA) is an immunoassay for quantitative screening of melamine.[2] It is based on the recognition of melamine by antibodies. Calibrators with known melamine concentrations, sample extracts, and Melamine horseradish peroxidase (HRP) conjugate are added to test wells coated with melamine antibody. Melamine from samples and Melamine HRP conjugate compete for binding to the melamine antibody, and subsequent washing removes unbound melamine and HRP conjugate. Substrate is then added to wells, and color develops in the wells proportional to the amount of bound enzyme conjugate present. After incubating, the reaction is stopped and the absorbance of the wells is read using a microplate reader. Sample melamine concentrations are interpolated from the calibrator standard curve.

Absorbance microplate readers from Molecular Devices are used to detect the Melamine ELISA. Data collection and analysis are performed using SoftMax® Pro GxP Software, an industry-standard analysis software for FDA 21 CFR Part 11 compliance. A preconfigured protocol to simplify data collection and analysis for Abraxis Melamine ELISA kit is available in SoftMax Pro Software.

Materials

- Molecular Devices microplate reader with absorbance detection mode:
  - SpectraMax® Plus 384 (cat. #PLUS 384)
  - SpectraMax 190 (cat. #190)
  - SpectraMax 340PC384 (cat. #340PC 384)
  - VersaMax™ (cat. #VERSAMAX)
  - SpectraMax M2/M2e (cat. # M2 or M2E)
  - SpectraMax M5/M5e (cat. #M5 or M5E)
  - FlexStation® 3 (cat. #FLEX3)
  - SpectraMax i3x (cat. #i3x)
  - SpectraMax iD3 (cat. #iD3)
- Melamine ELISA kit (Abraxis cat. #50005B)

100 µL of Stop Solution was added to each well using a multichannel pipettor.

The absorbance of the wells was read on a VersaMax microplate reader at 450 nm using a preconfigured protocol in the SoftMax Pro Software.

Calibrators were plotted using the 4-parameter curve fit in SoftMax Pro Software.

Benefits

- Quality assurance screening solution for the detection of melamine contamination
- Maximum throughput with minimum manual plate handling
- Complete data analysis with SoftMax Pro GxP Software

Methods

Reagents were equilibrated to room temperature prior to running the assay. The 5x Wash Buffer Concentrate was diluted 1:5 in deionized water to make wash solution.

Test wells were placed into a micro well holder. Unused wells were sealed in a plastic bag with dessicant.

100 µL of Calibrators were added to the test wells using a new pipet tip for each dispense. To demonstrate detection to as low as 10 parts per billion (ppb), an additional calibrator dilution was prepared by diluting the 20 ppb calibrator 1:2. 50 µL of Melamine HRP Conjugate were added to each well.

The plate was gently rotated for 60 seconds and then incubated at room temperature for 30 minutes.

Contents of the wells were aspirated, and wells were washed with 400 µL of wash solution a total of 4 times.

Following the last wash, the plate was inverted onto absorbent paper to remove the last of the wash solution.

100 µL of Substrate (Color Solution) was added to each well using a multichannel pipettor.

The plate was incubated at room temperature for 20 minutes.

Results

Calibrators were plotted using a 4-parameter curve fit in SoftMax Pro Software. A logit/log curve fit may also be used, but 4-parameter is preferred. Figure 1 shows the standard curve plotted as $\%B/B_0$ vs. concentration, where B is mean absorbance value for each standard and B_0 is the mean absorbance of the zero standard. $\%B/B_0$ of samples will yield levels in ppb of melamine by interpolation from the standard curve. For this assay, 50% B/B_0 was calculated as 193 ppb, consistent with the range of values shown in the melamine ELISA kit product insert.

To demonstrate assay sensitivity down to 10 ppb, an additional standard was prepared by diluting the 20 ppb standard 1:2. The resulting graph of average optical density (OD) vs. concentration is shown in Figure 2. The stated assay sensitivity for the melamine ELISA kit is 10 ppb.

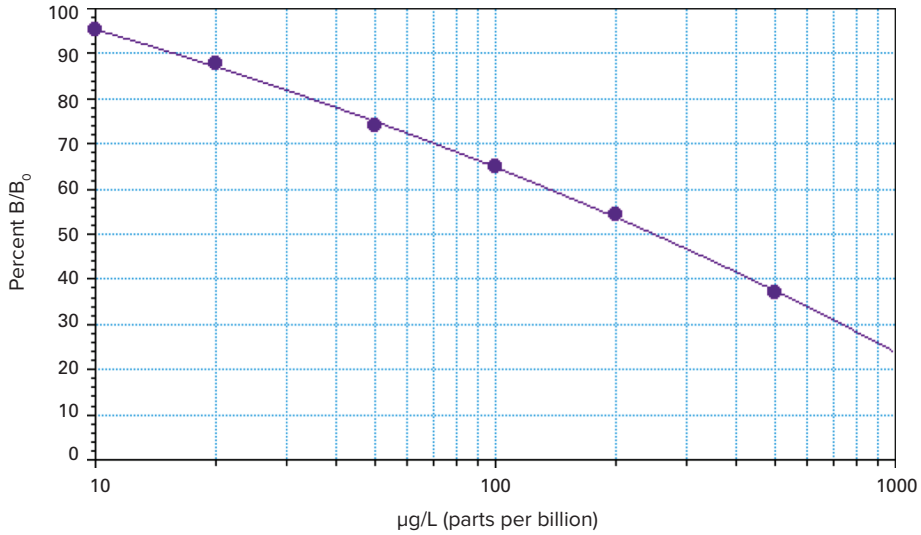

Figure 1. Melamine standard curve. Melamine standards plotted as $\%B/B_0$ vs. concentration (r2 = 0.999).

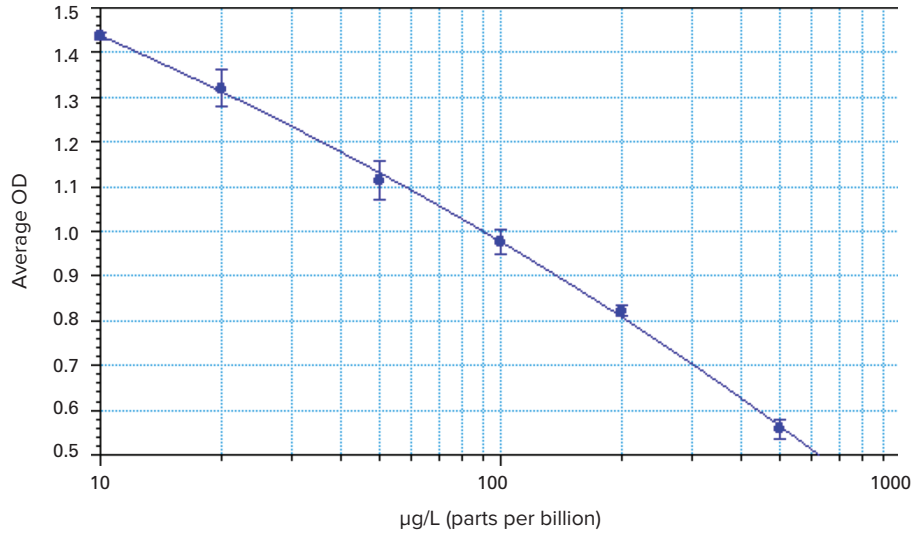

Figure 2. Melamine assay sensitivity. Melamine standard curve demonstrating assay sensitivity down to 10 ppb.

Melamine concentrations at least as low as 10 ppb can be reliably detected using the VersaMax reader. Similar results are obtained with the EMax microplate reader (data not shown).

According to performance data for test reproducibility presented in the melamine ELISA kit product insert, coefficients of variance (CVs) for standards are < 10%. In this application note we demonstrate CVs of < 5% for all standards. (See Table 1.)

µg/L (ppb)	Average OD	Std Dev	%CV	$\%B/B_0$
500	0.558	0.021	3.8	37
200	0.827	0.012	1.5	55
100	0.997	0.027	2.8	65
50	1.113	0.044	3.9	74
20	1.321	0.042	3.2	88
10	1.437	0.008	0.5	95

Table 1. Melamine standards. Average, standard deviation, CV, and $\%B/B_0$ were calculated by SoftMax Pro Software. CVs range from 0.8 to 4.9, well below the 10% test reproducibility limit for standards indicated by Abraxis.

Conclusion

The test sensitivity for the melamine ELISA is 10 ppb (µg/L). We have demonstrated that the VersaMax Microplate Reader can detect down to at least 10 ppb melamine. The middle of the test (50% B/B_0), where determinations give the most accurate results, is 193 ppb. This is consistent with values stated by Abraxis. CVs were shown to be less than 5% for all standards, well below the test reproducibility guideline of < 10% indicated in the melamine ELISA product insert.

The melamine ELISA kit from Abraxis, together with SpectraMax, VersaMax, VMax, and EMax microplate readers from Molecular Devices, offer a quality assurance screening solution for the detection of melamine contamination in such food products as milk, infant formula, pet food, and confectionery. Molecular Devices also offers a mass spectrometry-based solution for food safety testing through its joint venture partnership with Applied Biosystems. Complete data analysis is provided by SoftMax Pro GxP Software, an industry-standard analysis software for FDA 21 CFR Part 11 compliance.

For increased throughput, SpectraMax microplate readers can be integrated with Molecular Devices StakMax® Microplate Handling System. Users may also configure the StakMax system with Molecular Devices AquaMax® 4000 Microplate Washer. These combinations maximize throughput while minimizing manual plate handling.

References

1. Garber, E. A. (2008) Detection of Melamine Using Commercial Enzyme-Linked Immunosorbent Assay Technology. *J. Food Protection* 71(3): 590-594.
2. Abraxis Melamine Plate Kit (Cat. #50005B) Instructional Booklet.

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