

# High-Throughput Melamine Detection with SpectraMax Absorbance Microplate Readers and Beacon Analytical Systems' Melamine Plate Kit

SPECTRAMAX APPLICATION NOTE #20



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## 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 contamination can cause serious illness or death in animals and humans, there is increased interest in identifying methods for detecting melamine contamination in a variety of food products. In this application note, the Melamine Plate Kit from Beacon Analytical Systems is introduced and validated.

The Beacon Melamine Plate Kit is a competitive enzyme-linked immunosorbent assay (ELISA) for the quantitative analysis of melamine in contaminated samples. A variety of sample types, including dairy products, wheat gluten, and wet and dry pet foods, may be assayed using this kit. Melamine is extracted from a sample by blending or shaking with extraction solution. Melamine HRP conjugate, sample extract, and calibrators are then pipetted into test wells coated with melamine antibody. After a 30-minute incubation, wells are washed to remove unbound melamine, and substrate is added to wells. The reaction is stopped after 30 minutes, and the absorbance of the wells is measured using an absorbance microplate reader. The melamine concentrations of test samples are interpolated from a melamine standard curve.

Absorbance microplate readers from Molecular Devices are used to detect the absorbance in the melamine assay. Molecular Devices' AquaMax® 4000 Microplate Washer aspirates and dispenses all microplate wells simultaneously for rapid completion of the required wash steps. Data collection and analysis are performed using a melamine ELISA preconfigured protocol in

SoftMax® Pro GxP Software, an industry-standard analysis software for FDA 21 CFR Part 11 compliance.

## MATERIALS

- Molecular Devices microplate reader with absorbance detection mode:
  - SpectraMax® Plus<sup>384</sup> (Cat. #PLUS 384)
  - SpectraMax 190 (Cat. #190)
  - SpectraMax 340PC<sup>384</sup> (Cat. #340PC 384)
  - VersaMax™ (Cat. #VERSAMAX)
  - VMax® (Cat. #VMAX)
  - EMax® (Cat. #EMAX)
  - SpectraMax M2/M2<sup>e</sup> (Cat. # M2 or M2E)
  - SpectraMax M5/M5<sup>e</sup> (Cat. #M5 or M5E)
  - FlexStation® 3 (Cat. #FLEX3)
- Molecular Devices' AquaMax 4000 microplate washer (configure to order)
- Melamine Plate Kit (Beacon Analytical Systems, Cat. #20-0158)

## METHODS

The following test procedure was used:<sup>1</sup>

- Reagents were allowed to reach room temperature prior to running the test.
- The appropriate number of test wells coated with melamine antibody were placed in the microwell holder. Remaining test wells were re-sealed in the zippered bag provided. Additional non-antibody-coated wells were placed in the microwell holder so that all 96 positions were occupied; this allowed for use of the AquaMax 4000 microplate washer, which washes all 96 wells at once.
- 150 µL of Calibrators (in triplicate) or diluted sample extract were added to the appropriate test wells using a pipet with disposable tips. A clean pipet tip was used for each.

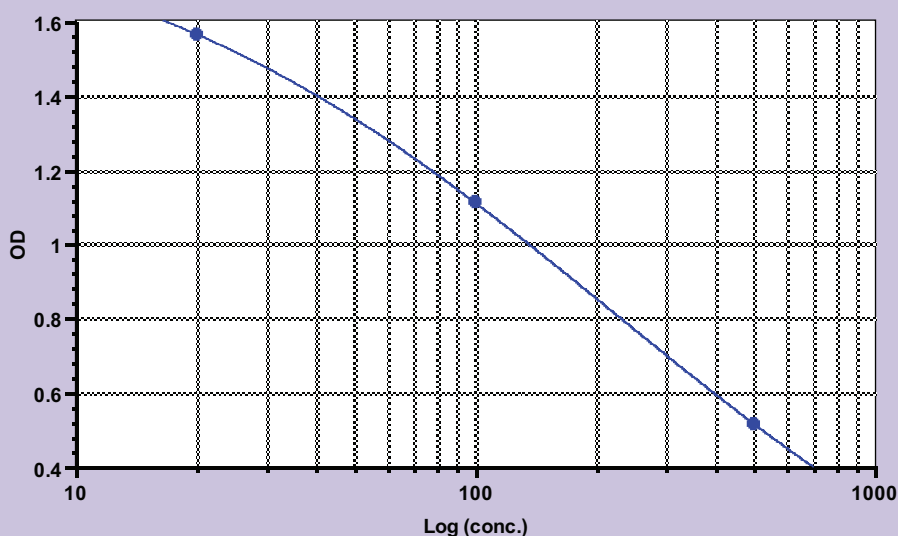
- 50  $\mu$ L of Melamine HRP conjugate was added to each well.
- The plate was shaken gently for 60 seconds and incubated at room temperature for 30 minutes.
- Contents of the wells were aspirated and 250  $\mu$ L of wash solution (1x PBS/0.05% Tween-20) was dispensed using the AquaMax 4000 Microplate Washer, for a total of four aspirate/dispense cycles.
- Following the last wash, the inverted plate was tapped onto absorbent paper to remove any residual wash solution.
- 100  $\mu$ L of Substrate was dispensed into each test well, and the plate was incubated at room temperature for 30 minutes.
- 100  $\mu$ L of Stop Solution was dispensed into each test well.
- The absorbance of the test wells was read on a VersaMax Microplate Reader at 450 nm using a pre-configured protocol in the SoftMax Pro Software.
- Calibrators were plotted using SoftMax Pro Software.

## RESULTS

Optical density (OD) values were used to construct a standard curve. Calibrators were plotted as OD values of the calibrators (y-axis) versus the log of the calibrator concentrations (x-axis). Sample melamine content could then be interpolated from the standard curve. Samples with OD values greater than the lowest calibrator or less than the highest calibrator must be reported as < 20 ppb or > 500 ppb, respectively.

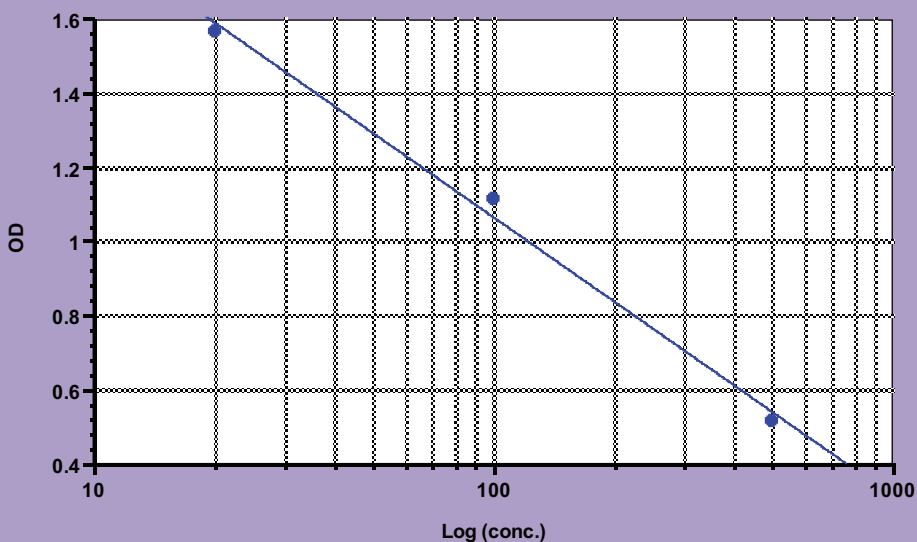
Figure 1 shows the melamine standard curve plotted as described above, with a 4-parameter curve fit. Figure 2 shows the melamine standards plotted using a semi-log curve fit. The 4-parameter curve typically gives a better fit at

**Melamine Standard Curve with 4-Parameter Fit (Figure 1)**



Melamine standards at 20, 100, and 500 ppb plotted as optical density of calibrators (OD) versus the log of the calibrator concentration. A 4-parameter curve fit was used.  $r^2 = 1.000$ .

**Melamine Standard Curve with Semi-Log Curve Fit (Figure 2)**



Melamine standards at 20, 100, and 500 ppb plotted as optical density of calibrators (OD) versus the log of the calibrator concentration. Here, a semi-log curve fit was used.  $r^2 = 0.994$ .

the high and low ends of the standard curve and is preferable.

Table 1 summarizes the Melamine Plate Kit Calibrator data. Excellent CVs of less than 5% were observed using the test procedure outlined in the product insert, and the AquaMax® 4000 microplate washer for the required wash steps.

| <b>Table 1. Melamine Plate Kit Data Summary</b> |                   |                |            |
|---|-------------------|----------------|------------|
| <b>Calibrator (ppb)</b>                         | <b>Average OD</b> | <b>Std Dev</b> | <b>%CV</b> |
| 0   | 1.916             | 0.032          | 1.7        |
| 20  | 1.565             | 0.062          | 4.0        |
| 100   | 1.112             | 0.033          | 3.0        |
| 500   | 0.516             | 0.024          | 4.6        |

Average OD, standard deviation, and %CVs were calculated by SoftMax Pro Software. Standards were run in triplicate.

## CONCLUSION

The Melamine Plate Kit from Beacon Analytical Systems, 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 food products such as milk, milk powder, yogurt, wheat gluten, and moist and dry pet food. The AquaMax 4000 Microplate Washer from Molecular Devices enables automation of the ELISA wash steps for excellent reproducibility and can be integrated with the StakMax® Microplate Handling System for increased throughput. Users may also configure the StakMax system with SpectraMax and VersaMax microplate readers in order to minimize manual plate handling. Complete data analysis is provided by SoftMax Pro GxP Software, an industry-standard analysis software for FDA 21 CFR Part 11 compliance.

## REFERENCES

1. Melamine Plate Kit Instructional Booklet (Beacon Analytical Systems)

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