

APPLICATION NOTE

High-throughput melamine detection with Romer Labs AgraQuant Melamine Assays 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 contamination can cause serious illness or death in animals and people, there is increased interest in identifying methods for detecting melamine contamination in a variety of food products. In this application note, two melamine ELISA assay kits from Romer Labs are introduced and validated: the AgraQuant Melamine Assay and the AgraQuant Melamine Sensitive Assay.

The AgraQuant Melamine Assay is a direct competitive enzyme-linked immunosorbent assay (ELISA) for the quantification of melamine and is intended for use with wheat gluten and pet food.¹ The AgraQuant Melamine Sensitive Assay is a more sensitive ELISA that has been validated for milk, milk powder, and other dairy products.² For both assays, test samples and enzyme-conjugated melamine are dispensed into microwells that have been pre-coated with melamine antibody. The test samples and standards compete with enzyme-conjugated melamine for binding to the antibody. Following incubation, the microwells are washed and substrate added, followed by a second incubation. After the second incubation, the reaction is quenched using the Stop Solution, and the absorbance of the mixture is measured at 450 nm using a microplate reader. The color intensity of the mixture is inversely

proportional to the amount of melamine present in the standards or samples. The melamine concentrations of test samples are interpolated using a melamine standard curve.

Absorbance microplate readers from Molecular Devices are used to detect the absorbance in the melamine assays. 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, including:
 - SpectraMax® Plus 384 (cat. #PLUS 384)
 - SpectraMax 190 (cat. #190)
 - SpectraMax 340PC384 (cat. #340PC 384)
 - VersaMax™ (cat. #VERSAMAX)
 - SpectraMax M2/M2^e (cat. # M2 or M2E)
 - SpectraMax M5/M5^e (cat. #M5 or M5E)
 - FlexStation® 3 (cat. #FLEX3)
 - SpectraMax i3x (cat. #i3x)
 - SpectraMax iD3 (cat. #iD3)
- AgraQuant Melamine Test Kit (Romer Labs cat. #COKAQ9300)
- AgraQuant Melamine Sensitive Test Kit (Romer Labs cat. #COKAQ9400)

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

For both kits, the following procedure was used:

Reagents were equilibrated to room temperature prior to use.

Test wells were placed into a microwell strip holder. Unused wells were sealed in a plastic pouch with desiccant.

150 µL of melamine standards were added to test wells with a single-channel pipette, using a new tip for each dispense. The Melamine Assay kit includes 0, 20, 100, and 500 parts per billion (ppb) standards, while the Melamine Sensitive Assay kit includes 0, 20, 100, and 1000 ppb standards.

50 µL of conjugate were dispensed into each well using an 8-channel pipette.

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 deionized water a total of four times.

Following the second wash, the plate was inverted onto absorbent paper to remove the remaining wash solution.

100 µL of Substrate was added to each well using a multi-channel pipette. The plate was incubated at room temperature for 20 minutes.

100 µL of Stop Solution was added to each well using a multi-channel pipette.

The absorbance of the 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 a semi-log curve fit in the SoftMax Pro Software.

Results

Optical density (OD) values expressed as a percentage of the OD value of the zero ppb standard (B/B_0) were used to construct a semi-log response curve. Sample melamine content can be interpolated from the standard curve. If a sample contains greater than 500 ppb (regular kit) or 1000 ppb (sensitive kit), the sample should be further diluted and re-analyzed to obtain an accurate result. If a sample contains melamine below the 20 ppb standard, the result should be reported as "< (20 x dilution factor) ppb".^(1, 2)

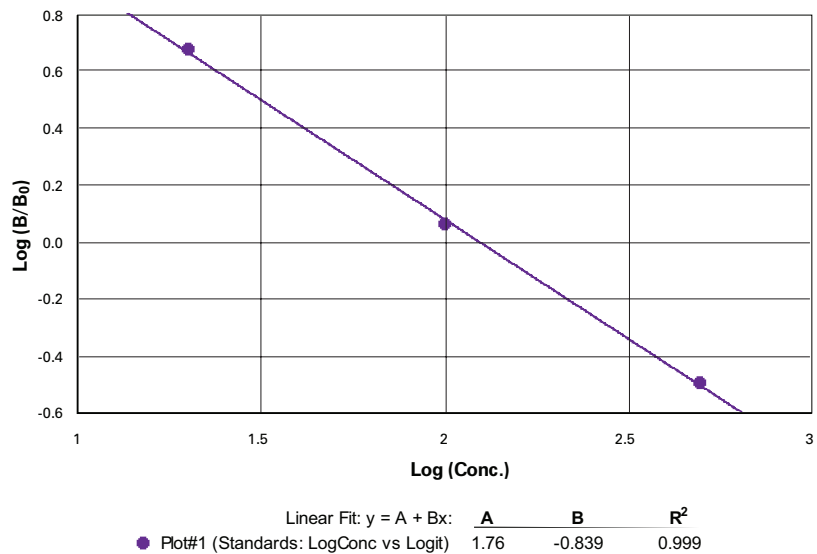


Figure 1. Melamine test kit standard curve. Melamine standards at 20, 100, and 500 ppb plotted as $\text{logit}(B/B_0)$ vs. $\log(\text{concentration})$. $r^2 = 0.999$. 50% inhibition = 124.2 ppb.

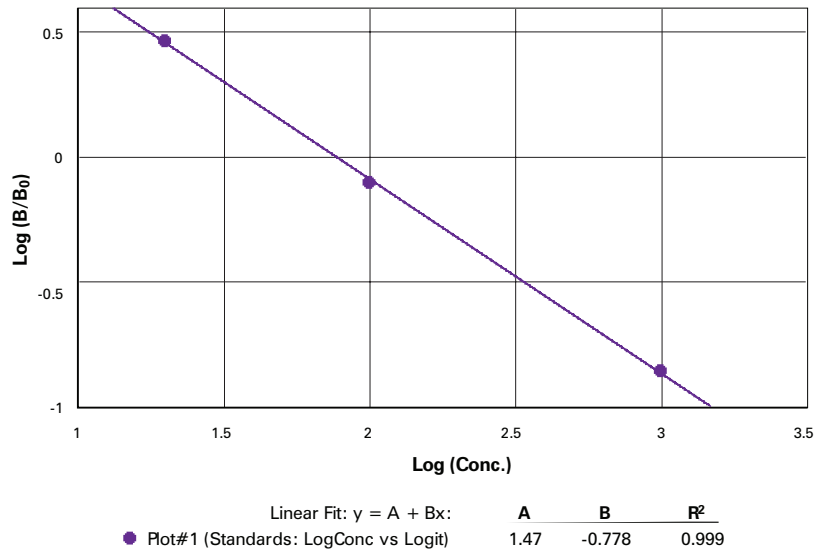


Figure 2. Melamine sensitive test kit standard curve. Melamine standards at 20, 100, and 1000 ppb plotted as $\text{logit}(B/B_0)$ vs. $\log(\text{concentration})$. $r^2 = 0.999$. 50% inhibition = 77.4 ppb..

Figure 1 shows the standard curve for the Melamine Assay plotted as $\logit(B/B_0)$ vs. \log (concentration), where B is the mean absorbance value for each standard and B_0 is the mean absorbance of the 0 ppb standard. Figure 2 shows the standard curve for the Melamine Sensitive Assay. The r^2 values were 0.999 for each standard curve, satisfying Romer Labs recommendation that the r^2 be no less than 0.985 for the Melamine Assay and no less than 0.990 for the Melamine Sensitive Assay. 50% inhibition was calculated as 124.2 for the Melamine Assay and 77.4 ppb for the Melamine Sensitive Assay; both values were comparable to those shown in the certificate of performance for each kit

According to data for test reproducibility presented in the Melamine Test Kit and Melamine Sensitive Test Kit certificates of performance, coefficients of variance (CVs) for standards are <9%. In this application note we demonstrate CVs of $\leq 5\%$ for all standards. (See Table 1 and Table 2.).

Conclusion

The Melamine Test Kit and Melamine Sensitive Test Kit from Romer Labs, 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. Complete data analysis is provided by SoftMax Pro GxP Software, an industry-standard analysis software for FDA 21 CFR Part 11 compliance.

The AquaMax® 4000 Microplate Washer from Molecular Devices enables automation of the ELISA wash steps

Standard (ppb)	Average OD	Std Dev	B/B ₀ , &	CV, %
20	0.872	0.020	82.6	2.3
100	0.563	0.022	53.3	3.9
500	0.255	0.007	24.2	2.8

Table 1. Melamine test kit. Average OD, standard deviation, average B/B₀, and CVs were calculated by SoftMax Pro Software. CVs $\leq 5\%$ compare favorably to CVs <9% shown in the certificate of performance provided by Romer Labs for each test kit.

Standard (ppb)	Average OD	Std Dev	B/B ₀ , &	CV, %
20	0.948	0.047	74.6	5.0
100	0.560	0.001	44.1	0.1
1000	0.155	0.001	12.2	0.8

Table 2. Melamine sensitive test kit. Average OD, standard deviation, average B/B₀, and CVs were calculated by SoftMax Pro Software. CVs $\leq 5\%$ compare favorably to CVs <9% shown in the certificate of performance provided by Romer Labs for each test kit.

and can be integrated with Molecular Devices StakMax® Microplate Handling System for increased throughput. Users may also configure the StakMax system with Molecular Devices SpectraMax and VersaMax microplate readers. These combinations maximize throughput while minimizing manual plate handling.

References

1. AgraQuant Melamine Test Kit product insert and Certificate of Performance.
2. AgraQuant Melamine Sensitive Test Kit product insert and Certificate of Performance.

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