Quality Control (QC) Criteria

- The correlation coefficient (R^2) of the calibration curve, analyzed using a 4-parameter logistic regression, must be ≥ 0.99.
- The average absorbance of the zero calibrator replicates must be ≥ 1.0.
- The average absorbance of calibrator replicates must have a coefficient of variation (%CV) < 15%.
- The average absorbance of sample replicates must have a coefficient of variation (%CV) < 20%.

Result Interpretation

It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculations is available upon request. Please contact Beacon for further details.

To ensure the validity of the results, please adhere to the following:

- Ensure QC criteria are met.
- The concentration of Melamine in a sample is determined by comparing the average sample absorbance to the standard curve. This value must then be multiplied by the dilution factor used.
- In the event that the average absorbance of the sample is lower than the highest calibrator, further dilute the sample extract in 10% methanol/20 mM PBS to fit into the standard curve and retest alongside the calibrators. Sample results must be multiplied by the total dilution factor used.

Technical Assistance

For questions regarding this kit or for additional information about Beacon products, contact us.

Beacon Analytical Systems, Inc.

82 Industrial Park Rd. Saco, ME 04046

Tel. +1-207-571-4302

info@beaconkits.com | www.beaconkits.com

Safety

Stop Solution is 1N hydrochloric acid. Handle with care. To receive complete safety information on this product, contact Beacon Analytical Systems, Inc., and request Safety Data Sheets.

General Limited Warranty

Beacon Analytical Systems, Inc. (“Beacon”) warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product’s printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty, or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Beacon’s sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

Melamine Plate Kit Product Brochure

Catalog No. 20-0158

Rev. B

Intended Use

The Beacon Melamine Plate Kit is an immunoassay for the detection of Melamine in contaminated samples. This product is intended for research use only.

Principles

Calibrators and the Sample Extract(s) are pipetted into the mixing wells followed by Melamine HRP Enzyme Conjugate. The reagents are mixed and transferred to the test wells to initiate the reaction. During an incubation, Melamine in the calibrator/sample and Melamine HRP Enzyme Conjugate compete for binding to the polyclonal Melamine antibody immobilized on the test wells surface. Following the incubation, the wells are washed to remove any unbound Melamine and Melamine HRP Enzyme Conjugate. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following an incubation, the reaction is stopped with the addition of Stop Solution and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the Melamine concentration of the sample is derived.

Reagents and Materials Provided

1 Unit	Plate containing 12 test strips of 8 wells each that are vacuum sealed in an aluminized pouch with a desiccant.
1 Unit	Plate containing 12 strips of 8 mixing wells each that are packaged in a zip-loc bag.
4 X 3 mL	Vials of Melamine Calibrators (0, 20, 100, and 500 ppb).
1 X 7.5 mL	Bottle of Melamine HRP Enzyme Conjugate.
1 X 50 mL	Bottle of 10X Wash Concentrate (dilute prior to use).
1 X 14 mL	Bottle of Substrate.
1 X 14 mL	Bottle of Stop Solution.

Reagents and Materials Required but Not Provided

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Multichannel pipette(s) (8 channels) with disposable tips capable of dispensing the required volume(s).
- Laboratory quality distilled or deionized water.
- Reagents and materials for sample preparation.
- Materials for 1X wash solution preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Timer.
- Microtiter plate or strip reader capable of reading at 450 nm.

Kit Handling Notes and Precautions

- Read the product brochure in its entirety prior to use.
- The kit, in its original packaging, can be used until the end of the month indicated on the box label.
- Do not use reagents after expiration date.
- Store all kit components at 2°C to 8°C (36°F to 46°F) when not in use.
- Reagents should be brought to room temperature, 20°C to 28°C (68°F to 82°F), prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Running Calibrators and Samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume is critical to obtain proper assay results.
- If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Substrate and Stop Solution.
- All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.
- Each reagent is optimized for use in the Beacon Melamine Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Melamine Plate Kits with different lot numbers.
- Do not reuse test wells.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Damage to or obstruction of the optical surface may cause unsatisfactory results.

Sample Preparation

Moist Cat Food: (Dilution Factor: 100)

1. Homogenize the sample using a blender.
2. Weigh 2 g of the homogenized sample into a clean container.
3. Measure 10 mL of 60% methanol and add to the container.
4. Vortex vigorously to mix.
5. Sonicate the sample for 1 minute.
6. Vortex the sample for 1 minute. Let stand for 5 minutes to allow layers to separate.
7. Transfer the clear, upper layer to a microcentrifuge tube.
8. Centrifuge for 5 minutes at 12,000 x g.
9. Filter the supernatant using a G6 glass filter into a clean vial.
10. Dilute the extract 1:20 in 10% methanol/20 mM phosphate buffered saline (PBS) and use in the assay.

Wheat Gluten: (Dilution Factor: 500)

1. Homogenize the sample.
2. 0.2 g of homogenized sample and add it to a container.
3. Measure 10 mL of 1N HCl/60% methanol and add it to the container.
4. Vortex and sonicate vigorously to dissolve the sample. Ensure no clumps remain in the solution before proceeding to the next step.
5. Dilute the extract 1:10 in 10% methanol/20 mM phosphate buffered saline (PBS).
6. Transfer the diluted extract to a microcentrifuge tube and centrifuge for 10 minutes at 12,000 x g.
7. Filter the supernatant using a G6 glass filter into a clean vial. Use the filtrate in the assay.

Dry Cat Food: (Dilution Factor: 200)

1. Homogenize the sample using a blender or coffee grinder.
2. Weigh 1 g of the homogenized sample into a clean container.
3. Measure 10 mL of 60% methanol and add to the container.
4. Vortex vigorously to mix.
5. Sonicate the sample for 1 minute.
6. Vortex the sample for 1 minute. Let stand for 5 minutes to allow layers to separate.
7. Transfer the clear, upper layer to a microcentrifuge tube.
8. Centrifuge for 5 minutes at 12,000 x g.
9. Filter the supernatant using a G6 glass filter into a clean vial.
10. Dilute the extract 1:20 in 10% methanol/20 mM phosphate buffered saline (PBS) and use in the assay.

1X Wash Solution Preparation

1. Measure 450 mL of laboratory quality distilled or deionized water and transfer to a clean container with a tight-fitting lid.
2. Transfer the contents of the 10X Wash Concentrate bottle to the container.
3. Gently swirl to mix.
4. Transfer the 1X Wash Solution to a wash bottle to use in the assay.

Assay Procedure

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Place the appropriate number of mixing wells and test wells into a holder. Be sure to re-seal unused test wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **180 µL of Calibrators and Sample Extract(s)** into the appropriate mixing well. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
4. Dispense **60 µL of Enzyme Conjugate** into each mixing well.
5. Mix the contents of each well by gently pipetting up and down with a multichannel pipette and transfer **200 µL of the mixture** to the test wells.
6. Gently shake the wells for 60 seconds using a back-and-forth motion and incubate for **30 minutes** at room temperature. Discard the mixing wells.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with 1X Wash Solution and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution. Alternatively, the last wash can be done using laboratory quality distilled or deionized water to reduce interference associated with wash solution bubbles.
8. Dispense **100 µL of Substrate** into each well.
9. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **30 minutes** at room temperature.
10. Dispense **100 µL of Stop Solution** into each well in the same order of addition as the Substrate.
11. Gently shake the wells for 30 seconds using a back-and-forth motion.
12. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each well at 450 nm using a plate or strip reader within 10 minutes of stopping the assay. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
13. Dispose of used test wells in an appropriate waste container.