CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: Sample containing less color than a calibrator will have a concentration of Melamine greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration less than the concentration of the calibrator.
2. Quantitative interpretation requires graphing the absorbances of the calibrators (Y axis) versus the log of the calibrator concentration (X axis) on semi-log graph paper. A straight line is drawn through the calibrator points and the sample absorbances are located on the line. The corresponding point on the X axis is the concentration of the sample. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 20 ppb or >500 ppb, respectively.

Alternatively, Beacon can supply a spreadsheet template which can be used for data reduction. Please contact Beacon for further details.

SAMPLE CALCULATIONS

Well Contents	OD	Average OD ± SD*	%RSD	%Bo**
Negative Control	1.728 1.744	1.736 ± 0.012	0.69	100
20 ppb Calibrator	1.427 1.391	1.434 ± 0.026	1.78	82.6
100 ppb Calibrator	0.855 0.858	0.856 ± 0.002	0.29	49.3
500 ppb Calibrator	0.341 0.335	0.338 ± 0.004	1.19	19.5
Sample	0.634 0.610	0.622 ± 0.017	2.7	35.8

Actual values may vary; this data is for example purposes only.
* standard deviation
** %Bo equals average sample absorbance divided by average negative control absorbance times 100%.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

SAFETY

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

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.

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Beacon Analytical Systems Inc.

Melamine plate Kit

Cat.# 20-0158

Instructional Booklet

Read Completely Before Use.

INTENDED USE

The Beacon Melamine Plate Kit is a competitive ELISA for the quantitative analysis of Melamine in contaminated samples.

ASSAY PRINCIPLES

The Beacon Melamine plate kit is a competitive enzyme-labeled immunoassay. Melamine is extracted from a sample by blending or shaking with extraction solution. The Melamine HRP conjugate, sample extract and calibrators are pipetted into the 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 wash with laboratory grade water, a clear substrate is then added to the wells and any bound enzyme conjugate causes the conversion to a blue color. Following a 30 minute incubation, the reaction is stopped and the amount of color in each well is read. The color of the unknown samples is compared to the color of the calibrators and the Melamine concentration of the samples is derived.

REAGENTS AND MATERIALS PROVIDED

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8ºC.

1 plate containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating dessicant.

4 vials each containing 3 mL of Melamine calibrators corresponding to 0, 20, 100 and 500 µg/L (ppb) of Melamine.

1 vial containing 7.5 mL Melamine HRP Enzyme Conjugate.

1 vial containing 14 mL of Substrate.

1 vial containing 14 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)

1 vial containing 50 mL of 10X PBST Wash Solution.

1 plate of red tabbed mixing wells.

1 Instructional Booklet

PRECAUTIONS

- 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.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Do not use reagents after expiration date.
- Reagents should be brought to room temperature, 20 – 28ºC (62 – 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Melamine is a toxic chemical and should be treated with care.
- The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.

MATERIALS REQUIRED BUT NOT PROVIDED

- Laboratory quality distilled or deionized water.
- Pipet with disposable tips capable of dispensing 50 µL.
- Multi-channel pipet; 8 channel capable of dispensing 50 and 100 µL.
- Paper towels or equivalent absorbent material.
- Microwell plate or strip reader with 450nm filter.
- Timer
- Wash solution and bottle. PBST wash solution:10mM PBS +0.05% Tween 20, pH 7.4.

SAMPLE PREPARATION

Moist cat food extraction protocol: Dilution factor: 100

1. Homogenize sample using a blender until it resembles a gritty pudding.
2. Extract 2 g of sample with 10 mL of 60% MeOH/water and vortex vigorously.
3. Sonicate sample for 1 minute.
4. Vortex sample again for 1 minute. Let stand for 5 minutes to allow layers to separate.
5. Centrifuge upper clear layer for 5 minutes at 10,000 rpm using Microcentrifuge.
6. Filter the clear upper phase with G6 glass filter and save the extract into a clean vial.
7. Dilute the crystal clear extract 1:20 into sample diluent (0.1 ml + 1.9 ml of 10% MeOH/20 mM PBS).
8. This diluted sample is ready for the assay.
9. ** This protocol was tested with 8 moist cat food samples (non-recalled) at 15 ppm Melamine spiked.
10. The recoveries were between 75-117 %.

Wheat gluten extraction Protocol: Dilution factor: 500

1. Homogenize or blend wheat gluten sample
2. Weigh 0.2 g* of homogenized sample and add 10 ml* of acidic 60% Methanol/water (1ml of 1N HCl /100 ml of 60% MeOH/water))
3. Vortex and sonicate vigorously to dissolve the sample; make sure no clumps are in the solution.
4. Dilute the extract 1:10 into sample diluent (0.5 ml +4.5 ml of 10% MeOH/20 mMPBS)
5. Centrifuge the upper clear layer for 10 min at 10,000 rpm using Microcentrifuge.
6. Filter the clear upper layer with G6 glass filter and save the filtrate into a clean vial.
7. This diluted sample is ready for the assay.
8. The wash solution for this application is PBST.

* A higher degree of adulteration needs more extraction solvent to ensure the extraction efficiency. Operators should use their own discretion. If the test results indicate a higher concentration of contamination, retest samples with double volume of extraction solvent (ex. 0.2g/20 ml).

Dry cat food extract: Dilution factor: 200

1. Homogenize dry cat food sample using a blender or coffee grinder.
2. Weigh 1 g of homogenized sample and add 10 ml of 60% Methanol/water.
3. Vortex vigorously and sonicate for 1 minute and votex again for 1 min and let it stand for 5 min.
4. Centrifuge the upper clear layer for 5min at 10,000 rpm using Microcentrifuge.
5. Filter the clear upper layer with G6 glass filter and save the filtrate into a clean vial
6. Dilute the extract 1:20 into sample diluent (0.1 ml + 1.9 ml of 10% MeOH/20 mM PBS)
7. This diluted sample is ready for the assay.

TEST PROCEDURE

TEST PROCEDURE (Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

1. Prepare the 1X wash solution by adding the contents of the 10X wash concentrate bottle to 450 mL Lab grade water in a wash bottle.
2. Allow reagents and sample extracts reach room temperature prior to running the test.
3. Place the appropriate number of test wells into a microwell holder. Be sure to re-seal unused wells in the zip-lock bag with desiccant.
4. Place the same number of mixing wells as test wells into a microwell holder. Be sure to re-seal unused wells in the zip-lock bag.
5. Using a pipet with disposable tips, dispense **180 µL of standards or diluted sample extracts** into the appropriate mixing well.
6. Add **60 µL of Enzyme conjugate** to all mixing wells.
7. Using a multichannel pipet, mix the contents by gently pipetting the solution in and out 4 or 5 times before transferring **200 µL of the sample/HRP mixture** into the test wells. Shake the plate gently for **60 seconds** and incubate the test wells for **30 minutes**.
8. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with wash solution and dump. Repeat 4X for a total of five washes.
9. Following the last wash tap the inverted wells onto absorbent paper to remove the last of the wash solution.
10. Dispense **100 µL of Substrate** into each well.
11. Incubate the wells for **30 minutes**.
12. Dispense **100 µL of Stop Solution** into each test well.
13. Read and record the absorbance of the wells at 450nm using a strip or plate reader.