Aflatoxin M1 Plate Kit
Cat.# 20-0201

Product Insert

READ COMPLETELY BEFORE USE.

INTENDED USE

The Beacon Aflatoxin M1 Plate Kit is a competitive ELISA for the quantitative analysis of Aflatoxin M1 in contaminated samples, such as fresh milk and other dairy products.

USE PRINCIPLES

The Beacon Aflatoxin M1 plate kit is a competitive enzyme-labeled immunoassay. Sample extract and calibrators are pipetted into the test wells followed by Aflatoxin M1 HRP enzyme conjugate to initiate the reaction. During this incubation Aflatoxin M1 from the sample and Aflatoxin M1 HRP conjugate compete for binding to the Aflatoxin M1 antibody. Following this incubation, the wells are washed to remove any unbound Aflatoxin M1 and Aflatoxin M1 HRP 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 another 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 Aflatoxin M1 concentration of the sample is derived. The color intensity is inversely proportional to the amount of Aflatoxin M1 present.

MATERIALS PROVIDED IN THE BEACON AFLATOXIN M1 PLATE KIT

- **Plate** – (1) containing 12 test strips of 8 wells coated each, vacuum-packed in aluminized pouch with indicating desiccant
- **Negative Control** – (1) vial containing 2 mL of 0.0 ppt (ng/L) Aflatoxin M1
- **Aflatoxin M1 Calibrators** – (5) vials containing 2 mL with a concentration of 2, 5, 10, 30, and 100 ppt (ng/L) of Aflatoxin M1
- **Aflatoxin M1 HRP Enzyme Conjugate** – (1) bottle containing 12 mL
- **Substrate** – (1) bottle containing 14 mL
- **Stop Solution** – (1) bottle containing 14 mL (Caution! Contains 1 N HCl. Handle with care.)
- **10X Wash Solution** – (1) bottle containing 50 mL (Must be diluted before use. See Assay Procedure Step 2.)

MATERIALS REQUIRED BUT NOT PROVIDED IN THE BEACON AFLATOXIN M1 PLATE KIT

- Microtiter plate reader or strip reader with 450 nm filter
- Clean running water or a wash bottle containing tap or deionized water
- Pipette with disposable tips capable of dispensing 50 µL
- Multi-channel pipette; 8 channel capable of dispensing 50 and 100 µL
- Paper towels or equivalent absorbent material
- Timer

- Wash bottle
- Vortex mixer
- Microcenrifuge and tubes (Ex. Eppendorf, centrifuge model 5415C)

SPECIFICITY

The Beacon Aflatoxin M1 Plate Kit can detect Aflatoxin M1 and M2. The percent cross reactivity (based on IC_{50}) of Aflatoxin M2 relative to Aflatoxin M1 is shown in the table below.

Compound	% Cross Reactivity
Aflatoxin M1	**100%**
Aflatoxin M2	**11%**

PRECAUTIONS

- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Each reagent is optimized for use in the Beacon Aflatoxin M1 Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Aflatoxin M1 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 to 28ºC (62 to 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Aflatoxin M1 is a toxin and should be treated with care.
- The Stop Solution is 1N hydrochloric acid, which is corrosive and an irritant. 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.
- Precise transfer of samples and reagents by using an appropriate and calibrated pipette is critical to obtain proper assay results. Please pipette carefully.

SAMPLE PREPARATION

A) Dry milk powder

1. Weigh out 3 grams of dry powder milk into a clean container and add 30 mL of laboratory grade water.
2. Vortex gently for 2 minutes to dissolve the powder completely.
3. Transfer 1.2 to 1.4 mL (per tube) of the reconstituted milk sample into two clean microcentrifuge tubes. Centrifuge for 5 minutes at 12,000 X g.
4. Carefully transfer the middle liquid layer from the two tubes into a clean glass test tube. Avoid pipetting the top lipid layer. Mix the contents from the two vials well by vortexing.
5. The sample is now ready to be tested in the assay.

B) Fresh milk (raw milk)

Start the sample preparation from step 3 in the dry milk powder sample preparation.

ASSAY PROCEDURE

1. Allow reagents and sample extracts to reach room temperature prior to running the assay.
2. Prepare the 1X wash solution by adding the contents of the 10X wash concentrate to 450 mL labratory grade water in a wash bottle.
3. Place the appropriate number of test wells into a micro well holder. Be sure to re-seal any unused wells in the zip-lock bag with desiccant.
4. Using a pipette with disposable tips, add **100 µL of the Calibrators or sample extract** into the appropriate test wells. Be sure to use a clean pipette tip for each.
5. Shake the plate gently for 30 seconds using a back and forth motion. Then incubate the wells for **30 minutes** at room temperature. **Do not wash wells before adding the HRP Enzyme Conjugate**.
6. Add **100ul of HRP Enzyme Conjugate** to each well.
7. Shake the plate gently for 30 seconds using a back and forth motion. Then incubate the wells for **15 minutes** at room temperature.

8. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with 1X wash solution and then decant. 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 the Substrate** into each well. Shake the plate gently for 30 seconds using a back and forth motion.
11. Incubate the wells for **30 minutes** at room temperature.
12. Dispense **100 µL of the Stop Solution** into each well.
13. Measure and record the absorbance (Optical Density; OD) of the wells at 450 nm. If the plate reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
14. If the absorbance of a sample is lower than the highest calibrator (100 ppt), the concentration of Aflatoxin M1 is too high and out of range of the standard curve. Dilute the sample in laboratory grade water and rerun. Samples should be diluted to fit into the standard curve (2 ppt to 100 ppt). Results must then be multiplied by the dilution factor used.

CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: Samples containing less color than a calibrator well have a concentration of Aflatoxin M1 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. It is preferred that quantitative results from the ELISA are determined using commercially available software using a 4-Parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-Parameter software is not available. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 2 ppt or > 100 ppt, respectively. 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	2.07 2.05	2.06 ± 0.033	1.60	100
2 ppt Calibrator	1.88 1.87	1.88 ± 0.004	0.23	90
5 ppt Calibrator	1.53 1.52	1.52 ± 0.007	0.43	73
10 ppt Calibrator	1.14 1.12	1.13 ± 0.014	1.27	54
30 ppt Calibrator	0.59 0.62	0.60 ± 0.021	3.49	29
100 ppt Calibrator	0.33 0.33	0.33 ± 0.003	0.90	16

Actual values may vary; this data is for example purposes only.
* standard deviation
** $\%B_0$ equals average sample absorbance divided by average Negative Control absorbance multiplied by 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 Safety Data Sheets. Stop Solution is 1N hydrochloric acid. Handle with care.

General Limited Warranty

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