Aflatoxin M1 ULTRA ELISA Quantitative

For the quantitative detection of Aflatoxin M1 in milk, skim milk powder, and yogurt.

Kit Components

- 96 Antibody-coated Microwells
- Mixing wells
- Aflatoxin M1 Standards
- Ready to Use Conjugate
- M1 Free Skim Milk
- Substrate Reagent
- Stop Solution
- PBS-T Powder

Catalog No: 961AFLM01C-ULTRA

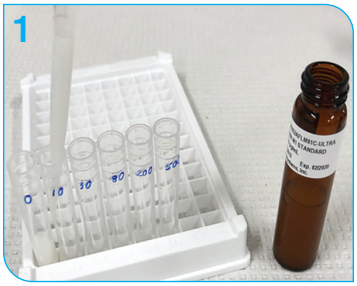
Required Equipment Not Supplied with Kit

- Single or multi-channel pipettor with 10, 100, 200 and 1000 µL tips
- Microtubes
- Wash bottle
- Absorbent paper towels
- Centrifuge (and tubes)
- Microplate reader with 450 nm filter
- Triton X-100 (20% in deionized water, v/v)
- Yogurt diluent for yogurt sample (Cat# 937YOG001)

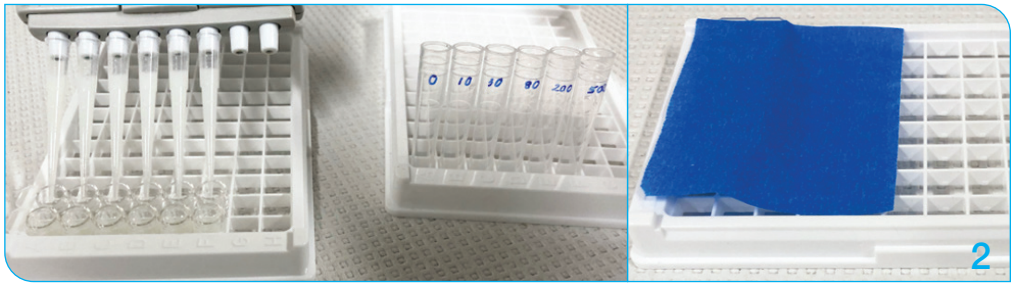
Reagents Provided

1x Pouch	Antibody coated microwell plate		96 wells (12 eight-well strips) in a microwell holder coated with a mouse anti-aflatoxin monoclonal antibody, *Ready-To-Use*
1x Plate	Mixing wells	Red	96 non-coated wells (12 eight-well strips) in a microwell holder, *Ready-To-Use*
6x Vials	Aflatoxin M1 standards	Black cap	8.0 mL/vial of Aflatoxin M1 at the following concentrations: 0.0, 5.0, 15.0, 50.0, 150.0, 500.0 pg/mL (ppt), *Ready-To-Use*
1x Bottle	Aflatoxin HRP-conjugate	Green cap	12 mL of Aflatoxin conjugated to horseradish peroxidase in buffer with preservative, *Ready-To-Use*
1x Bottle	Substrate reagent	Blue cap	12 mL stabilized tetramethylbenzidine (TMB), *Ready-To-Use*
1x Bottle	Stop solution	Red cap	12 mL Acidic Solution, *Ready-To-Use*
1x Pouch	Washing buffer		PBS with 0.05% Tween20®, bring to 1 liter with distilled water and store refrigerated.
1x Bottle	M1 free skim milk	White cap	12 mL of skim milk, *Ready-To-Use*

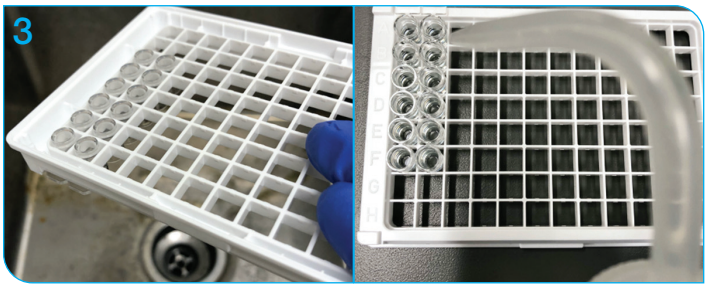
Assay Procedure



1 Transfer 1.2 mL of each standard and sample into microtubes. If running singlets, scale the volume down accordingly.

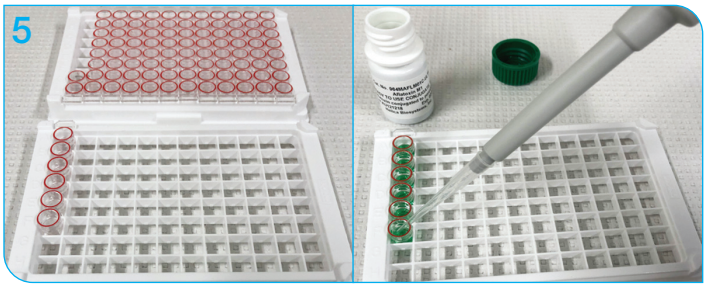


2 Use a multichannel pipettor to transfer 200 µL aliquots of standards and samples from the microtubes into the Antibody Coated Wells and seal plate. Incubate for 20 minutes as the first incubation.



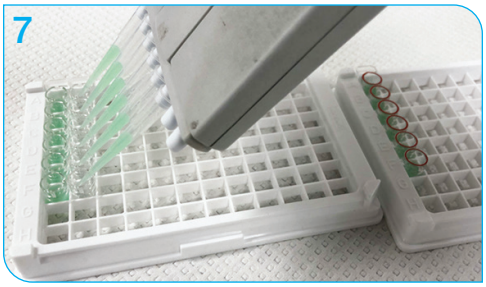
3 Empty the contents from the Antibody Coated Wells and discard into basin. Wash the wells by filling with the reconstituted PBS-Tween wash buffer, empty into basin, and repeat for a total of 3 washings. Tap the wells (face down) on a layer of absorbent paper.

4 **Repeat step 2.** Transfer 200 µL aliquots of standards and samples. Incubate for 20 minutes as the second incubation. Refer to photos and instructions in step 2.



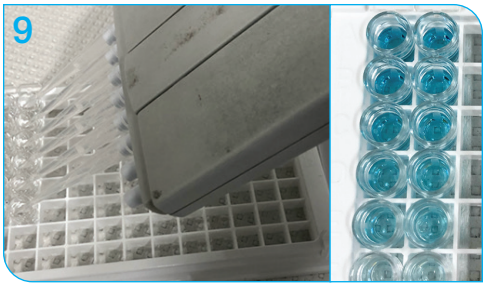
5 During the second incubation, dispense 150 µL of standard or sample from the microtube into the red mixing wells provided by the kit and add 150 µL of the conjugate to each red mixing well. Mix by priming pipettor at least 3 times. If running singlets, scale the volume down accordingly. *Note: Operator must record the location of each Standard and Sample throughout test.*

6 After the second incubation, wash the plate by repeating step 3. Refer to photos and instructions in step 3.



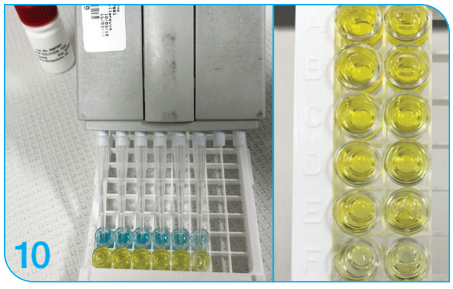
7 Transfer 100 µL of the conjugate mixture from each mixing well in step 5 to a corresponding Antibody Coated well. Seal and incubate for 20 minutes.

8 **Repeat step 3.** Refer to photos and instructions in step 3 for a total of **5 washings**.



9 Add 100 µL of enzyme substrate (TMB) to each Antibody Coated well and incubate for 10 minutes. Cover to avoid direct light. A blue color will develop.

10 Use a multi-channel pipettor to transfer 100 µL of stop solution to the Antibody Coated wells. The blue color will change to yellow.



11 Read the optical density of each microwell with a microplate reader at 450 nm using an air blank or a differential filter of 630 nm.