

Product Manual

AgraQuant® Aflatoxin M₁ High Sensitivity 5/100 ELISA kit **Article number 10002120/10002121**

Intended use

The AgraQuant® Aflatoxin M₁ High Sensitivity 5/100 ELISA kit is an immunoassay designed for the quantitative analysis of the presence of aflatoxin M₁ in milk and milk products. This product is intended for laboratory use.

Performance characteristics

Limit of detection (LOD): 2.9 ppt (raw milk)
4.2 ppt (fresh milk)
2.3 ppt (UHT milk)
33.3 ppt (milk powder)

Range of quantification: 5-100 ppt (milk)
50-1000 ppt (milk powder)

Plate format: 96 (10002120) or 48 wells (10002121)

Assay time: 75 minutes

About Aflatoxin M₁

Aflatoxins are highly toxic and carcinogenic compounds produced as metabolites by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Four principal types of aflatoxins are B₁, B₂, G₁, and G₂, each named for their distinct fluorescent properties. Aflatoxin M₁ is a potent hepatocarcinogen produced by hydroxylation of aflatoxin B₁. It is formed in the liver of animals following the ingestion of aflatoxin B₁ and is excreted mainly in milk. Processing of milk does not lead to a significant degradation of aflatoxin M₁, which can therefore occur in dairy products such as milk powder and cheese. Exposure of infants and children to aflatoxin M₁ is of particular concern, given their higher susceptibility to aflatoxins.

Product information

About AgraQuant® Aflatoxin M₁ High Sensitivity 5/100 ELISA kit

The AgraQuant® Aflatoxin M₁ High Sensitivity 5/100 test kit is a direct competitive enzyme-linked immunosorbent assay (ELISA) that quantifies the presence of aflatoxin M₁ in a given sample. It is specifically tailored for testing milk and milk products.

Storage information

Upon receipt, immediately transfer the AgraQuant® Aflatoxin M₁ High Sensitivity 5/100 ELISA kit to refrigerated storage conditions and keep it at 2-8° C (35-46°F) when not in use. Do not freeze. Do not use the kit beyond the expiration date indicated on the label.

Contents of the kit

The AgraQuant® Aflatoxin M₁ High Sensitivity 5/100 ELISA kit contains the following items:

10002120

- 96 antibody-coated microwells (12 eight-well strips) in a microwell holder sealed in a foil pouch
- 6 vials each with 1.5 mL of aflatoxin M₁ standard (0, 5, 10, 25, 50 and 100 ppt)
- 1 green-capped bottle with 15 mL of aflatoxin M₁-conjugate solution
- 1 blue-capped bottle with 15 mL of substrate solution
- 1 red-capped bottle with 15 mL of stop solution
- 1 white-capped bottle with 25 mL wash solution (20x concentrated)

10002121

- 48 antibody-coated microwells (6 eight-well strips) in a microwell holder sealed in a foil pouch
- 6 vials each with 0.75 mL of aflatoxin M₁ standard (0, 5, 10, 25, 50 and 100 ppt)
- 1 green-capped bottle with 7.5 mL of aflatoxin M₁-conjugate solution
- 1 blue-capped bottle with 7.5 mL of substrate solution
- 1 red-capped bottle with 7.5 mL of stop solution
- 1 white-capped bottle with 25 mL wash solution (20x concentrated)

Materials required but not included

Extraction procedure:

- Analytical balance with a weighing capacity up to 200 g
- Graduated cylinder with a minimum capacity of 100 mL
- Test tubes
- Centrifuge
- Vortex
- Distilled or deionized water
- Whatman #1 filter paper, or equivalent
- Filter funnel

Assay procedure:

- Calibrated 8-channel and single-channel pipettes with 100 µL disposable plastic tips
- Timer
- Wash bottle
- Distilled or deionized water
- Absorbent paper towels
- 3 reagent boats for use as reagent containers for an 8-channel pipette
- Microwell reader with 450 nm and 630 nm filters

Visit www.romerlabs.com or get in touch with your Romer Labs technical sales representative to find out which of these items are also available from Romer Labs®.

AgraQuant® Aflatoxin M₁ High Sensitivity 5/100 ELISA kit – assay principle

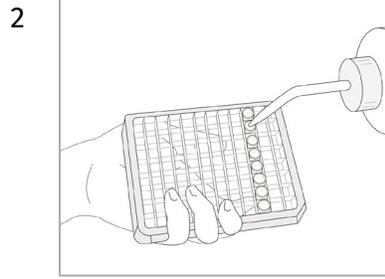
The AgraQuant® Aflatoxin M₁ High Sensitivity 5/100 test kit is a direct competitive enzyme-linked immunosorbent assay (ELISA). The samples or aflatoxin M₁ standards are added to the antibody-coated microwells, so that the aflatoxin M₁ is allowed to bind to the antibody binding sites. After a washing step, the enzyme-conjugated aflatoxin is added and allowed to bind to the free binding sites of the antibodies. After a second washing step, the enzyme substrate is added, which results in color development. The intensity of the color is inversely proportional to the concentration of aflatoxin M₁ in the sample or standard. A stop solution is then added, which changes the color from blue to yellow. The absorbance of each well is then measured at 450 nm and with a differential filter at 630 nm. The measurement must take place within 10 minutes after adding the stop solution. To analyze the results, please refer to the “Results analysis” section at the end of this product manual.

Protocol at a glance

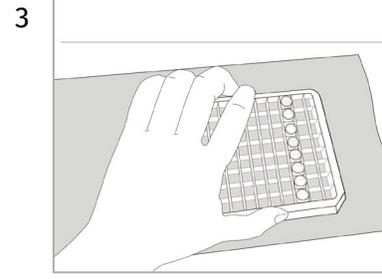
This section offers a brief overview of the AgraQuant® ELISA procedure. It is essential to read the complete product manual thoroughly before initiating the assay.



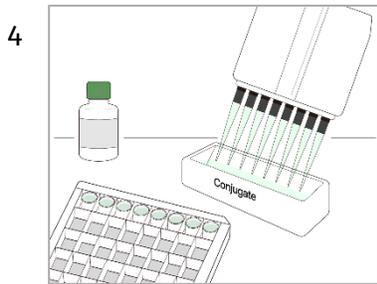
Pipette **100 µL of sample or standards** into the antibody-coated wells. **Incubate at RT for 45 minutes.**



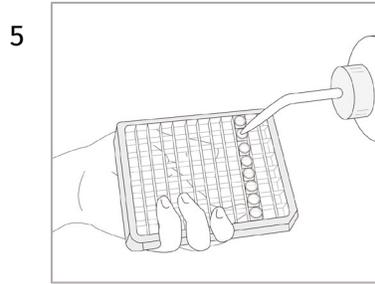
Wash 5 times with wash buffer



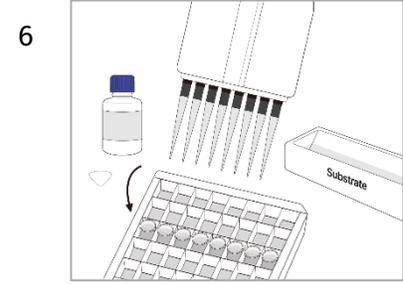
Tap dry the washed wells.



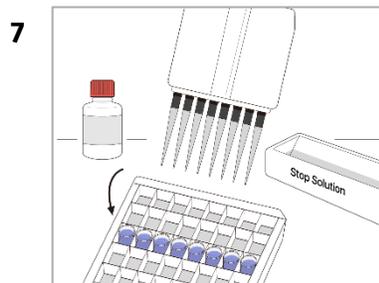
Pipette **100 µL of conjugate solution** into the antibody-coated wells. **Incubate at RT for 15 minutes.**



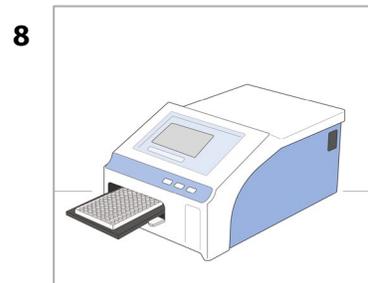
Repeat the washing steps.



Pipette **100 µL of substrate solution** into the antibody-coated wells. **Incubate at RT for 15 minutes.**



Pipette **100 µL of stop solution** into the antibody-coated wells.



Read the absorbance of each well at **450 nm** with a differential filter at **630 nm**.

Reagent and sample preparation

Sample preparation

Milk

1. Pipette 5 ml of milk sample (full-cream milk or skim milk) into a test tube and incubate for 30 minutes at 4°C.
2. Centrifuge the sample at 3000 g for 10 minutes.
3. Take 100 µL of milk serum below the fat layer.
4. Samples are ready for testing. Please read the ELISA procedure section and carefully follow the protocol.

Milk powder

1. Weigh 1 g of skim or full-cream milk powder into a 50 mL centrifuge tube.
2. Dissolve the milk powder with 10 mL of distilled or deionized water, preheated at 50°C.
3. Homogenize it using a vortex for 30 seconds.
4. Filter the sample with Whatman #1 filter paper or equivalent.
5. Collect the filtered milk solution.
6. Samples are ready for testing. Please read the ELISA procedure section and carefully follow the protocol.

Wash buffer preparation

Transfer the 25 mL of wash buffer 20x concentrated to a 500 mL plastic squeeze bottle. Add 475 mL distilled or deionized water and mix.

ELISA procedure

Before starting

Procedural guidelines:

- Make sure you have everything you need ready before starting the assay.
- All reagents and kit components must be allowed to reach room temperature, i.e. 18-30 °C (64-86 °F), before use.
- Run a standard curve with each assay.
- Adhere to the incubation times stated in the procedure. Use of incubation times other than those specified may return inaccurate results.
- We strongly recommended that you perform the assay with an 8-channel pipette.
- Do not run more than 6 eight-well strips in one experiment when using an 8-channel pipette. If an 8-channel pipette is not used (i.e. using only single channel pipettes), we recommend that you run no more than a total of 16 samples and standards (2 test strips) in any one experiment.
- Do not return unused reagents into their original bottles.

Precautions:

- Store reagents at 2-8°C (35-46°F) when not in use, and do not use beyond the expiration date.
- The stop solution contains acid. Avoid contact with skin or eyes. If exposed, flush with water.
- Treat all materials, containers and devices that are exposed to the sample or standards as if they were contaminated with toxin.
- Wear protective gloves and safety glasses when using the kit.
- Dispose of all single-use materials, containers and devices appropriately after use.

Assay protocol

1. Place an appropriate number of **antibody-coated microwells** in a microwell strip holder. Return unused microwells to the foil pouch with the desiccant packet and reseal pouch with tape.
2. Using a single channel pipette, add **100 µL of each standard or sample** into the antibody-coated wells. Use a fresh pipette tip for each standard or sample.
Note: Make sure the pipette tip has been completely emptied.
3. Incubate at room temperature for **45 minutes**.
4. Empty the content of the microwell strips into a waste container. **Wash** by filling each microwell with wash solution, and then dump the wash solution from the microwell strips. Repeat this step 4 times for a total of 5 washes.

Note: Take care not to dislodge the strips from the holder during the washing steps.

5. Lay several layers of absorbent paper towels on a flat surface and tap the microwell strips on towels to remove as much residual wash solution as possible after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.

Note: Never insert absorbent paper directly into the wells.

6. Measure the required amount of conjugate solution from the green-capped bottle (~120 μL /well or 1 mL/strip) and place it in a separate container (e.g. reagent boat when using the 8-channel pipette). Using an 8-channel pipette, dispense **100 μL of conjugate solution** into each antibody-coated microwell.

Note: Do not attempt to mix the content of the microwells by shaking the plate as this may cause well-to-well contamination.

7. Incubate at room temperature for **15 minutes**.

8. **Wash** by repeating the steps 4 and 5.

9. Measure the required amount of substrate solution from the blue-capped bottle (~120 μL /well or 1 mL/strip) and dispense it into a separate container (e.g. reagent boat for an 8-channel pipette). **Pipette 100 μL of the substrate solution** into each microwell using an 8-channel pipette. Incubate at room temperature for **15 minutes**.

10. Measure the required amount of stop solution from the red-capped bottle (~120 μL /well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipette). **Pipette 100 μL of stop solution** into each microwell using an 8-channel pipette. The color should change from blue to yellow.

11. Read the absorbance of each well within 10 minutes after the addition of the stop solution at **450 nm** (reference wavelength 630 nm) with a microwell reader.

Note: Carefully remove any air bubbles prior to reading the absorbance as they may affect the result.

Note: Do not return unused reagents to their original bottles. Carefully note which rows/strips contain standards or samples during the assay.

Results analysis

Results can be easily calculated using the **Romer Labs® spreadsheet** that is provided free of charge upon request. With the Romer Labs® spreadsheet you only need to insert the obtained OD values for standards and samples. The spreadsheet applies the Log/Logit regression model to construct a calibration curve. The correlation coefficient (R) of the calibration curve must be between -0.990 to -1.000. The aflatoxin concentration in your samples is calculated automatically by interpolation with the calibration curve. Alternatively, construct a dose-response curve using either the unmodified OD values of the standards or the OD values expressed as a percentage of the OD of the zero (0) standard. If the Log/Logit regression model is used for results interpretation, the correlation coefficient (R) of the calibration curve must be between -0.990 and -1.000.

When working according to the *sample preparation* section described in this product manual, the following dilution factors need to be applied for calculating the aflatoxin M₁ concentration in the samples:

- For milk: 1
- For milk powder: 10.9

Note: An OD value of less than 0.5 absorbance units for the 0 ppt standard may indicate the deterioration of reagents.

Technical support

Not sure if the test works with your specific samples or matrices? Let our longstanding experience in mycotoxin testing work for you. Contact your Romer Labs technical sales representative for more information.

Visit www.romerlabs.com to find worldwide contact information.
For further information please contact us at:

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