Version: v19
Date: 01.2026

Product Manual

AgraQuant® Aflatoxin M_1 Sensitive 25/500 ELISA kit
Article number 10002116/10002117

Intended use
The AgraQuant® Aflatoxin M1 Sensitive 25/500 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): 18 ppt (fresh milk)
252 ppt (skim milk powder)
257 ppt (full-cream milk powder)
128 ppt (cheese)

Range of quantification: 25-500 ppt (fresh milk)
270-5400 ppt (skim/full cream milk powder)
200-4000 ppt (cheese)

Plate format: 96 (10002116) or 48 wells (10002117)

Assay time: 80 minutes

About aflatoxin M_1
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 M_1 Sensitive 25/500 ELISA kit

The AgraQuant® Aflatoxin M_1 Sensitive 25/500 test kit is a direct competitive enzyme-linked immunosorbent assay (ELISA) that quantifies the presence of aflatoxin M_1 in a given sample. It is specifically tailored for testing in milk and milk products.

Storage information

Upon receipt, immediately transfer the AgraQuant® Aflatoxin M_1 Sensitive 25/500 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 M_1 Sensitive 25/500 ELISA test kit contains the following items:

10002116

- 96 antibody-coated microwells (12 eight-well strips) in a microwell holder sealed in a foil pouch
- 96 non-coated dilution microwells (12 white eight-well strips)
- 6 vials each with 1.5 mL of aflatoxin M1 standard (0, 25, 50, 100, 200 and 500 ppt)
- 1 green-capped bottle with 25 mL of aflatoxin-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 30 mL of sample diluent
- 1 white-capped bottle of with 25 mL wash solution (20x concentrated)

10002117

- 48 antibody-coated microwells (6 eight-well strips) in a microwell holder sealed in a foil pouch
- 48 non-coated dilution microwells (6 white eight-well strips)
- 6 vials each with 0.75 mL of aflatoxin M1 standard (0, 25, 50, 100, 200 and 500 ppt)
- 1 green-capped bottle with 12.5 mL of aflatoxin-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 15 mL of sample diluent
- 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
- Volumetric flasks with stopper
- Centrifuge
- ACS grade methanol
- ACS grade dichloromethane (for certain commodities)
- ACS grade hexane (for certain commodities)
- PBS buffer (for certain commodities)
- Distilled or deionized water
- Magnetic stirrer or horizontal shaker
- Evaporation system

Assay procedure:

- Calibrated 8-channel and single-channel pipettes with 100 µL and 200 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®.

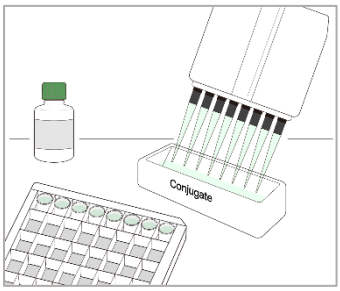
AgraQuant® Aflatoxin M_1 Sensitive 25/500 ELISA kit – assay principle

The AgraQuant® Aflatoxin M_1 Sensitive 25/500 test kit is a direct competitive enzyme-linked immunosorbent assay (ELISA). The extracted sample and enzyme-conjugated aflatoxin is mixed and added to the antibody-coated microwell. Aflatoxin M_1 in the samples or standards is allowed to compete with enzyme-conjugated aflatoxin for the antibody binding sites. After a 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 M_1 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.

1

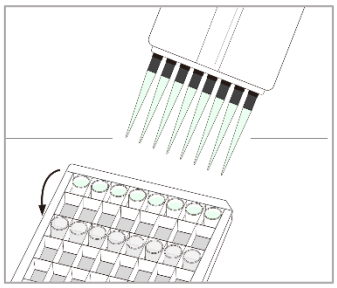


Pipette **200 µL of conjugate solution** into the dilution wells.

2

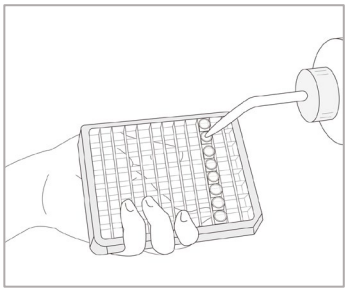
Add **100 µL of each standard or sample extract** into the dilution wells.

3



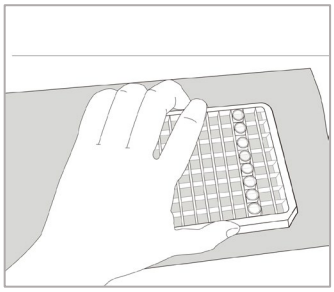
Mix well and **transfer 100 µL** from the dilution wells into the antibody-coated wells. **Incubate at RT for 60 minutes.**

4



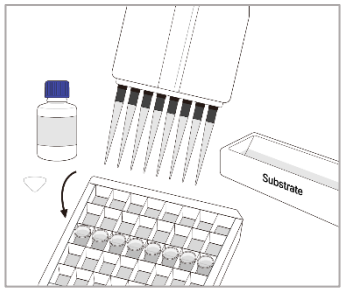
Wash **5 times** with wash buffer.

5



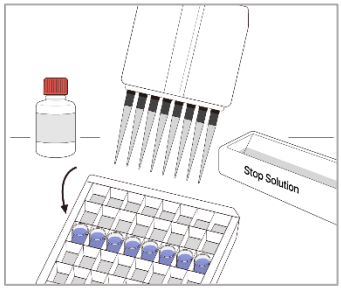
Tap dry the washed wells.

6



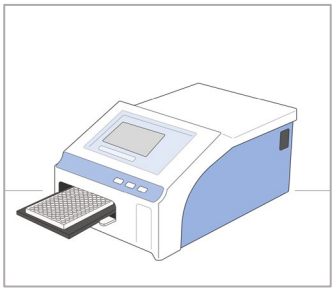
Pipette **100 µL of substrate** solution into the antibody-coated wells. **Incubate at RT for 20 minutes.**

7



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

8



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 fresh milk sample (full-cream milk or skim milk) into a test tube and incubate for 30 minutes at 4°C.
2. Optional: Centrifuge the sample at 3000 g for 10 minutes.
3. Take 400 µL of milk serum below the fat layer and mix with 100 µL of 100% methanol (the ratio between milk serum and methanol is 4:1).
4. Samples are ready for testing. Please read the *ELISA procedure* section and carefully follow the protocol.

Milk Powder

1. Weigh 10 g of skim or full-cream milk powder into a flask.
2. Dissolve the milk powder with 100 mL of distilled or deionized water, preheated at 50°C.
3. Homogenize it by using a magnetic stirrer.
4. Follow the above-mentioned sample preparation method for milk.

Cheese

1. Weigh 2 g of cheese sample into a flask and add 40 mL of dichloromethane.
2. Extract for 30 minutes by shaking at 260 rpm in a rotary shaker at room temperature.
3. Take 5 mL of extract and evaporate at 60°C under a nitrogen stream or using an evaporation system.
4. Re-dissolve the residue in 2 mL of a solution 1:1:2 of PBS:methanol:hexane (0.5 mL of PBS (Phosphate-buffered saline), 0.5 mL methanol, 1 mL hexane).
5. Take 0.2 mL of the aqueous/methanolic phase (lower layer) and dilute it with 0.3 mL of sample diluent.
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.
In case some precipitate is formed during storage, the concentrate should be warmed up at 37°C for 15 minutes before dilution.

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 recommend that you perform the assay with an 8-channel pipette.
- If you use a one channel pipette, we recommend that you run not more than a total of 16 samples and standards (2 test strips) on one experiment. We strongly recommend that you not run more than 6 eight-well strips in one experiment when using an 8-channel pipette.
- 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 them 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 the appropriate number of **white dilution wells** in a microwell strip holder. One dilution well will be required for each standard (i.e. 0, 25, 50, 100, 200 and 500 ppt) or sample.
2. Place an equal number of **antibody-coated microwells** in a microwell strip holder. Return unused microwells to the foil pouch with the desiccant packet and reseal pouch.
3. Measure the required amount of conjugate solution from the green-capped bottle (~240 µL/well or 2 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 **200 µL of conjugate solution** into each dilution well.
   ➲ **Did you know?** Ratio of conjugate to standard/sample should remain at 2:1, but the volumes of conjugate and standards/samples can be reduced, e.g. using 100 µL and 50 µL, respectively. The content to be transferred from dilution wells to antibody-coated wells must remain at 100 µL.
4. Using a single channel pipette, add **100 µL of each standard or sample** into the dilution wells containing 200 µL of conjugate. Use a fresh pipette tip for each standard or sample.

   Note: Make sure the pipette tip has been completely emptied.

5. Using an 8-channel pipette with fresh tips for each 8-well strip, mix each well by carefully pipetting up and down 3 times and immediately **transfer 100 µL of the content** of each dilution well into a corresponding antibody-coated microwell. Incubate at room temperature for **60 minutes.**

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

6. Empty the content of the microwell strips into a waste container. **Wash** by filling each microwell with distilled or deionized water, and then dumping the water 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.

7. Lay several layers of absorbent paper towels on a flat surface and tap the microwell strips on towels to remove as much residual water 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.

8. 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 **20 minutes.**

9. 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.

10. 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 M_1 concentration in the samples:

- For milk: 1
- For milk powder: 10.8
- For cheese: 8

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:

Romer Labs® Division Holding GmbH
Technopark 5
3430 Tulln, Austria
T: +43 2272 61533
office.romerlabseu@dsm-firmenich.com