INSTRUCTIONS FOR USE

aokin mycontrol AFLA M1 IAC

Order No.: MYS-IC-31

Quantitative determination of **Aflatoxin M1**

Includes sample preparation with *aokinImmunoClean **M*** columns (IAC) designed for use with ***aokin*** **FP analyzer**.

Package content:

Order No. MYS-IC-31
aokin**mycontrol* ***AFLA M1 IAC

Order No	**Material**	**Content**	**Application**	**Storage**
MYS-IC-31-20 for 20 measurements	***aokin**mycontrol* ***AFLA M1 IAC*** Kit for quantitative determination of Aflatoxin M1 (AFLA M1)	***aokin**ImmunoClean **M AFLA M1*** ③, 20 units ***aokin** ReactionBuffer* ④, 1 bottle ***aokin**ICDilute**50x*** ⒹⓌ, 21 mL, 1 vial *sticker for **aokin** IC*Wash **AFLA M1*** ***AFLA M1** Reagent,* 1 unit ⑤ ***AFLA M1** Tracer,* 1 unit ⑤ ***AFLA M1** positive control, Standard **AFLA M1*** ⑤, ? µl, 1 unit	Measurement on ***aokin* FP analyzer**	Refrigerated Do not freeze
MYS-IC-31-100 for 100 measurements	***aokin**mycontrol* ***AFLA M1 IAC*** Kit for quantitative determination of Aflatoxin M1 (AFLA M1)	***aokin**ImmunoClean **M AFLA M1*** ③, 100 units ***aokin** EasyReaction Buffer Component A* ④Ⓐ, 1 unit ***aokin** EasyReaction Buffer Component B* ④Ⓑ, 1 unit *sticker for **aokin** EasyReactionBuffer* ***aokin**ICDilute**50x*** ⒹⓌ, 105 mL, 1 bottle *sticker for **aokin** IC*Wash **AFLA M1*** ***AFLA M1** Reagent,* 5 units ⑤ ***AFLA M1** Tracer,* 5 units ⑤ ***AFLA M1** positive control, Standard **AFLA M1*** ⑤, ? µl, 5 units	Measurement on ***aokin* FP analyzer**	Refrigerated Do not freeze

Analytical-kit for rapid and quantitative determination of Aflatoxin (AFLA) in food products as wheat, corn, animal feed, nuts, spices, cocoa and roasted coffee.

Materials

All materials provided are precisely weighed and calibrated. Control of the volume and concentration of the individual solutions are essential for the precision of the analysis.

Quality control:
All materials and reagents are prepared according to strict quality control protocols. Exchanging reagents between kits having different Lot-numbers will lead to erroneous results and is not permitted.

Preparation of buffers:

MYS-IC-31-20 for 20 measurements:
aokin *ReactionBuffer* ④ included in the kit has to be brought to room temperature (best 18-26°C) for usage.
Mix 20ml ***aokin****IC*Dilute50x ⒹⓌ with 980ml destilled or deionized water = a***okin***ICWash* **AFLA M1** Ⓦ Prepare the solution and label the container with the sticker st*icker for* ***aokin*** *IC*Wash **AFLA M1** included in the kit, on which you can note the preparation date.

MYS-IC-31-100 for 100 measurements:
The complete content of the *EasyReaction Buffer Component A* ④Ⓐ plus the *EasyReaction Buffer Component B* ④Ⓑ needs to be dissolved in a total of 500 mL destilled or deionized water = ***aokin*** *EasyReactionBuffer*. Prepare the solution and label the container with the sticker ***aokin*** *EasyReactionBuffer* included in the kit, on which you can note the preparation date. ***aokin*** *EasyReactionBuffer* ④ has to be brought to room temperature (best 18-26°C) for usage.
Mix 20ml ***aokin****IC*Dilute50x ⒹⓌ with 980ml destilled or deionized water = ***aokin****ICWash* ***AFLA M1*** Ⓦ Prepare 5x the solution and label the container with the sticker st*icker for* ***aokin*** *IC*Wash **AFLA M1** included in the kit, on which you can note the preparation date.

Storage and stability:

aokin *ReactionBuffer* ④ is stable for 12 months without refrigeration (15° - 30°C).

The diluted ***aokin****IC*Wash **AFLA M1** Ⓦ is stable for 12 month without refrigeration (15° - 30°C).

Kit reagents (***AFLA M1*** *Reagent*, ***AFLA M1*** *Tracer, positive control* ⑤) must be stored in a refrigerator (2-10°C). ***AFLA M1*** *positive control* is stable after dilution for only 1 day.

Any other components can be stored at ambient temperature.

Do not use expired or contaminated components, or components from other kits. Do not mix components from different manufactured lots. A certificate of analysis (COA) is available upon request at info@aokin.com.

Materials and instrumentation required but not provided:
aokin **FP analyzer**, 10x75 mm borosilicate glass test tubes for ***aokin*** **FP analyzer**, Micro-pipette: 10 – 100 µL, 100-1000 µL and related pipette tips, 2 mL collection tubes, tube vortex mixer, centrifuge, timer, vacuum-pump, vacuum manifold, glass fiber filters, ***aokin****ICadapter*, pH-indicator strips, deionized water for buffer reconstitution, methanol.

Alternative to the ***aokin*** **FP analyzer** other FP single tube readers or FP microplate readers can be used. Depending on instrumentation the applied volumes need adjustment. Black flat bottom microtiter plates are recommended for use with FP microplate readers.

Introduction

aokin*mycontrol* ***AFLA M1 IAC*** is a rapid method for quantifying Aflatoxin M1 (AFLA M1). It has been specifically designed and calibrated for the analysis of skimmed milk and other milk products, and includes a sample preparation with ImmunoClean columns (***aokin****ImmunoClean* ***M AFLA M1***). Samples in the µg/kg range (µg/kg = ppb) can be analysed for AFLA M1 in under 60 minutes.

aokin*mycontrol* ***AFLA M1 IAC*** is available with a calibration, which has been validated for food products. Daily checks of negative control and positive control (either liquid standard or reference matrix extract) are required to ensure reliable results.

FPA technology works on clear homogeneous liquids without particles or emulsions, therefore avoid practices that may contaminate the test controls, test buffer, or test reagents. ***aokin*** will gladly assist you customizing the test for your specific sample type and application. Please do not hesitate to contact us.

Polarization readings are affected by temperature. Polarization readings decrease by around 3 mP (millipolarization units) for a temperature increase of 4°C.
The method is best used at constant temperatures between controls and sample measures. If a temperature change of >10°C is observed, a new calibration is recommended.

Health:
All materials in this kit should be treated as any other laboratory chemicals. Avoid ingestion, eye contact and other potential detrimental exposure. A material safety data sheet (MSDS) is available upon request.

Negative controls:
Negative control should be run in triplicate at regular intervals with the single tube assay or on every microtiter plate, respectively. Avoid temperature variations of more than 1°C during one determination. Mix well with a vortexer and wait 60 s before taking the value. Be careful to avoid bubbles when pipetting into microtiter plates.

Negative control AFLA M1 IAC = ***Methanol***

Positive controls:
Positive controls are recommended as an additional measure to adjust the calibration for small temperature changes. Reference matrix materials are best used to determine the recovery rate and to do a recovery rate correction if needed.

Positive controls AFLA M1 IAC ⑤ = Standards diluted in ***Methanol*** Dilute included **Aflatoxin M1 300 ng/ml in acetonitrile** Lot:003001150120283 with **Methanol** (Dilutionfactor **1:20;** add 20µl of standard to 380µl Methanol, use 20 to 80µl within the assay); fill up with Methanol for overall 100µl sample volume (example: 50µl of 1:20 diluted positive control AFLA M1 + 50µl Methanol = 7.5ng/mL Aflatoxin M1 within the Assay)

Aflatoxin M1

Aflatoxins (AFLA) are mycotoxins produced by molds such as *Aspergillus flavus* and *Aspergillus parasiticus. Aflatoxin M1* is a potent hepatotoxic and hepatocarcinogenic mycotoxin, found in milk of cows, fed on meal contaminated with *Aflatoxin B1.* As a consequence, it is necessary to monitor AFLA content in food and feed products.

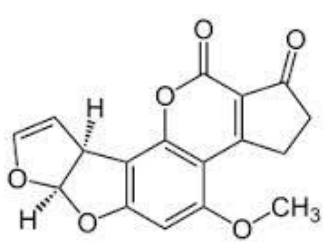


Figure 2: Chemical formula for Aflatoxin M1, $C_{17}H_{12}O_7$. Molecular weight: 328,27 g/mol

Standard sample preparation for corn (Page 9-12/12)
(for other types of samples follow the instructions on the last pages)

1. Preparation

1.1. Allow ***aokin****ReactionBuffer* to reach room temperature (15°C to 30°C) before use.

1.2. Prepare buffers and solutions:
Mix 20ml ***aokin****IC*Dilute50x ⒹⓌ with 980ml destilled or deionized water =
aokin*ICWash* ***AFLA M1*** Ⓦ
Positive control AFLA IAC:
Dilute included **Aflatoxin M1 300 ng/ml in acetonitrile** Lot:003001150120283
with **Methanol** (Dilutionfactor **1:20;** add 20µl of standard to 380µl Methanol, use 20 to 80µl within
the assay); fill up with Methanol for overall 100µl sample volume (example: 50µl of 1:20 diluted positive control AFLA M1 + 50µl Methanol = 7.5ng/mL Aflatoxin M1 within the Assay)

2. Instrument adjustment
Instruments must be set up and calibrated according to manufacturer's specifications. For detailed information, please consult instrument manual and contact technical support.

3. Sample collection, grinding and mixing
The analysis sample is collected, ground, and mixed/homogenised according to an approved procedure. For convenience volumes may be ground and mixed by using a knife-blender.

4. Sample
Measure 60 mL of fluid milk. Centrifuge the milk sample at greater than 15.000 x g for 15 minutes (in special centrifuge tubes).
Separate fat (top) layer from defatted (skim) layer. Use defatted (skim) milk for further analysis.

5. Filtration or centrifugation
Filter the raw extract through a fluted filter. Discard the filter cake and use the filtrate. Alternatively centrifuge the raw extract and use the supernatant.

6. Check pH value

6.1. Check the pH value. If necessary, readjust the pH value by adding diluted HCl or NaOH to a neutral pH (pH 6.5 - 7.5).

7. Clean up with *aokin*ImmunoClean *M* columns

7.1. **Preparation**
Attach the vacuum manifold to a pump. Attach the ***aokin****ImmunoClean* ***M*** ③ column to the vacuum manifold (or alternatively onto an ***aokin****ICAdapter in the case that the load is to be operated by means of gravity*) and transfer ***aokin****ICWash* **AFLA M1** Ⓦ solution onto the column. Connect the loading reservoir to the ***aokin****ImmunoClean* ***M*** ③ column. Pipette ***aokin****ICWash* **AFLA M1** Ⓦ into the loading reservoir.

Note: No air bubbles should be visible in the column. Let the ***aokin****ICWash* **AFLA M1** Ⓦ solution elute, leaving only a little liquid in the reservoir, so that the ***aokin****ICadapter* and ***aokin****ImmunoClean* ***M*** ③ column are still filled with liquid.

7.2. **Loading**
Load the column with 20 ml of the diluted filtrate. Adjust the vacuum so that a flow rate of approximately **1 ml/min** is produced (total running time approximately 20 minutes). The running speed can be reduced by closing the stopcock, or increased with applying vacuum. Alternatively, a positive pressure from above can be used.

Note: Do not let the ***aokin****ImmunoClean* ***M*** ③ column run dry before, or during loading.

Note: High flow rates lead to a reduced rate of recovery.

7.3. **Washing**

Fill 15 ml ***aokin****ICWash* **AFLA M1** Ⓦ into the loading reservoir. Adjust the vacuum so that a high flow rate is achieved (running time approximately 2 minutes). The column bed should appear relatively colorless. If this is not observed, the wash step may be repeated. Let the column run dry.

8. Elution

Place the ***aokin****ImmunoClean* ***M*** ③ column into a collection tube and centrifuge for 1 minute at 1000 x g to remove the residual liquid.

Set the ***aokin****ImmunoClean* ***M*** ③ column into a new clean collection tube and pipette 300 µl Methanol into the column, close (half closed) the column after that with the lid. When closing the column, about 100 µl Methanol will be **pushed through the gel**. After incubation for 3 minutes, centrifuge for 1 minute at 3000 x g. The liquid is present in the collection vessel after centrifugation.
Repeat the step, by placing the just used ***aokin****ImmunoClean* ***M*** ③ column into a new collection tube and pipette again 300 µl Methanol into the column, following the procedure. Unite the eluates of both collection tubes. Your sample is now ready for analysis.

Note: Eluates of some samples may show a precipitate. This leads to increased results. If you see or suspect a precipitation in the eluate than centrifuge eluate at high speed, than pipette supernatant into new collection tube and use for further analysis.

9. Introduction of general testing procedure

9.1. Pipette 960 µl of ***aokin****ReactionBuffer* ④ into the test tube.
9.2. Add 100 µl of sample extract (if necessery fill up with **Methanol** if sample eluat is too concentrated, example: 50 µl sample + 50 µl **Methanol** for overall 100 µl sample volume) or a control into the test tube.

Note: Run negative controls in triplicate, and positive control and samples as duplicates for best performance.

9.3. Add 20 µL of ***AFLA M1*** *Reagent (****BLUE*** *cap)* ⑤ into the test tube.
9.4. Mix well/vortex (without spilling).
9.5. Obtain background measurement readings of all samples and controls. Wait a minimum of 1 minute and a maximum of 15 minutes between mixing and reading.
9.6. Add 20 µL of ***AFLA M1*** *Tracer (****YELLOW*** cap) ⑤ into the first test tube.
9.7. Mix well.
9.8. Incubate for 60 seconds.
9.9. Obtain tracer measurement of the sample.
9.10. Repeat steps 9.6 to 9.9 for all test tubes.

Note: Include one set of control after each hour of testing, or on each microtiter plate. Performing measurements of negative controls regularly will ensure the accuracy of your determinations.

10. Data acquisition and analysis

10.1. Mix and Read Process

10.1.1. Mix buffer, sample and reagent in exact volumes and at exactly specified time intervals. Read background fluorescence in horizontal and vertical direction.
10.1.2. Add tracer, incubate for exactly the specified time. Read emission values in horizontal and vertical direction.

10.2. Background information

The fluorophore is excited with polarized light, depending on the rotational velocity of the fluorophore (or the fluorophore antibody complex) the emission will also be polarized. The emission intensities using horizontally directed and vertically directed polarization filters are detected for the background and for the reaction. Followed by an automatic calculation of polarization from the value changes in vertical and horizontal direction.
$P = (\Delta H - \Delta V)/ (\Delta H + \Delta V)$.

10.3. **Software: direct use of excel worksheet - *aokin mycontrol* version of software - alternatively use user interface**

Advice: Use a copy of the empty original excel worksheet to do all measurements of one series. When you start with a new series including negative controls, positive controls and sample analysis measurements, use a new copy of the empty original worksheet saved previously with the name of your series and the date.

Note:

- Connect computer with ***aokin*** FP analyser
- Install the ***aokin*** software
- One excel row = one experiment
- Green cells can be written into
- Yellow cells are for measuring: highlight a cell in the correct line and press F3
- Brown cells are for information only
- Light green cells contain evaluations = calculations and end results

11. Daily Check I:

a. Use negative control (= Methanol) in Step 9.2

b. Software: Choose *Daily Check I* in the user interface or alternatively *Offset at given T°C* Sheet in particular excel version of software

11.1. Pipette 960 µl of ***aokin****ReactionBuffer* ④ into a test tube.
11.2. Add 100 µl of a negative control into the test tube. The negative control for AFLA M1 IAC is Methanol.
11.3. Add 20 µL of ***AFLA M1*** *Reagent (**BLUE** cap)* ⑤ into the test tube.
11.4. Mix well/vortex (without spilling).
11.5. Obtain background measurement reading. Wait a minimum of 1 minute and a maximum of 15 minutes between mixing and reading.
11.6. Add 20 µL of ***AFLA M1*** *Tracer (**YELLOW** cap)* ⑤ into the first test tube.
11.7. Mix well.
11.8. Incubate for 60 seconds.
11.9. Obtain tracer measurement

Note: Run negative controls in triplicate.

12. Daily Check II-A:

a. Use liquid positive control in Step 9.2

b. Software: Choose *Daily Check II, Liquid standard* in the user interface or *Recovery rate at given T°C* Sheet in particular excel version of software

12.1. Pipette 960 µl of ***aokin****ReactionBuffer* ④ into a test tube.
Add 100 µl of a positive control into the test tube. The positive control is a liquid standard diluted in Methanol**.** Respectively use **20 – 80 µl** of the 1:20 diluted **aokin** *positive control* ⑤ (20µl 300ng/mL AFLA M1 in acetonitrile + 380µl Methanol). Fill up with 80 – 20µl **Methanol** for overall 100 µl sample volume (example: 50µl of 1:20 diluted positive control AFLA + 50µl Methanol = 7.5ng/mL Aflatoxin M1 within the Assay)
12.2. Add 20 µL of ***AFLA M1*** *Reagent (**BLUE** cap)* ⑤ into the test tube.
12.3. Mix well/vortex (without spilling).
12.4. Obtain background measurement reading. Wait a minimum of 1 minute and a maximum of 15 minutes between mixing and reading.
12.5. Add 20 µL of ***AFLA M1*** *Tracer (**YELLOW** cap)* ⑤ into the first test tube.
12.6. Mix well.
12.7. Incubate for 60 seconds.
12.8. Obtain tracer measurement

Note: Run positive controls in duplicate.

13. Alternative Daily Check II-B:
a. Use of final extract of a reference matrix material as positive control in Step 9.2
b. Software: Choose *Daily Check II, Reference Matrix* in the user interface or *Recovery rate at given T°C* Sheet in particular excel version of software

13.1. Choose a reference matrix sample most similar to your samples.
Example: In case you analyse wheat use a wheat reference matrix sample containing a known amount of mycotoxin.
13.2. Prepare the reference matrix sample according to Steps 4, 5, 6, 7, 8.
13.3. Pipette 960 µl of ***aokin****ReactionBuffer* ④ into a test tube.
13.4. Add 100 µl of the Reference Matrix extract into the test tube.
13.5. Add 20 µL of ***AFLA M1*** *Reagent (**BLUE** cap)* ⑤ into the test tube.
13.6. Mix well/vortex (without spilling).
13.7. Obtain background measurement reading. Wait a minimum of 1 minute and a maximum of 15 minutes between mixing and reading.
13.8. Add 20 µL of ***AFLA M1*** *Tracer (**YELLOW** cap)* ⑤ into the first test tube.
13.9. Mix well.
13.10. Incubate for 60 seconds.
13.11. Obtain tracer measurement

Note: Run positive controls in duplicate. Repeat with a different dilution (9.2), if needed.

14. Testing procedure – Sample:
a. Use sample extract in Step 9.2
b. Software: Choose *Analysis* in the user interface or *Analysis at given T°C* Sheet in particular excel version of software

14.1. Pipette 960 µl of ***aokin****ReactionBuffer* ④ into the test tube.
14.2. Add 100 µl of clear sample extract or a control into the test tube.
14.3. Add 20 µL of ***AFLA M1*** *Reagent (**BLUE** cap)* ⑤ into the test tube.
14.4. Mix well/vortex (without spilling).
14.5. Obtain background measurement readings of all samples and controls. Wait a minimum of 1 minute and a maximum of 15 minutes between mixing and reading.
14.6. Add 20 µL of ***AFLA M1*** *Tracer (**YELLOW** cap)* ⑤ into the first test tube.
14.7. Mix well.
14.8. Incubate for 60 seconds.
14.9. Obtain tracer measurement of the first sample.
14.10. Repeat steps 13.6 to 13.9 for all test tubes.

Note: Run samples as duplicates. Repeat with a different dilution (9.2), if needed.

15. Test validation

15.1. The mean negative control must read between 190 and 260 mP.
15.2. The positive control must always read lower than the negative control. Expected values of the positive control are 10 mP to 150 mP lower than the negative control.
15.3. If the negative or positive control produce values outside of the expected values, the instrument should be recalibrated using the procedures described in the instrument manual.
15.4. Check the accuracy of your measurements by regularly analysing reference materials (e.g. ***aokin****ReferenceMatrixMaterials*), or other verified materials with known amounts of contaminants. Participation in proficiency tests is recommended as good laboratory practice.

Recommendation: If you notice erroneous measurement values, change the test tube and repeat the measurement after performing measurement of controls. Check that the liquid in the tube is not cloudy and that there are no bubbles after mixing.

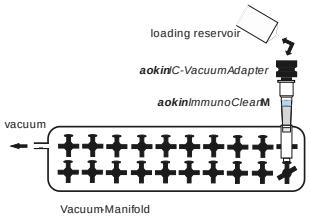
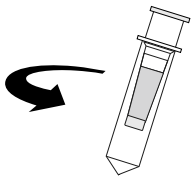
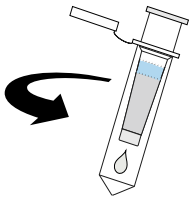
Note: Repeat the daily checks after each hour of testing, or on each microtiter plate. Performing measurements of controls regularly will ensure the accuracy of your determinations. The results of the daily checks are used to ensure the analytical performance.

Be aware: The general composition of controls and samples should always be identical in salt concentration, pH and solvent ratio.

Aflatoxin M1 / standard samples: ***Liquid Sample***

- Recommended for milk

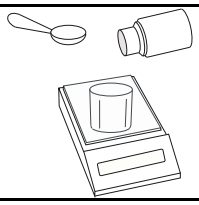
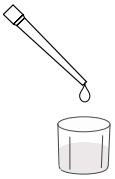
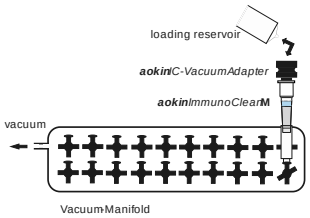
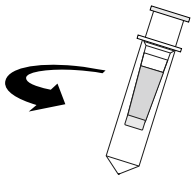
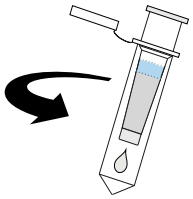
Procedure:

Extraction			(Centrifuge 15 minutes at 15.000 x g, use defatted layer)
		60 ml	skim milk sample
Purification			set up column
		50 ml	Load skimmed milk sample, 1 drop / second (slow flow rate)
		40 ml	wash column with ***aokin****ICWash* **AFLA M1** Ⓦ
		1 min	centrifuge at 1000 x g (waste tube)
		**Elution:**	
		400 µl	***aokin****ICElute* ***AFLA M1***
		3 min	incubation with lid on
		1 min	centrifuge at 3000 x g, use eluate for measurement
Measurement	Mix and read	**Quantification**	
			add into tube:
		960 µl	***aokin*** *ReactionBuffer* ④
		100 µl	sample
		20 µl	***AFLA M1*** *Reagent* ***BLUE*** ⑤
			mix well
			measure background
		20 µl	add ***AFLA M1*** *Tracer* ***YELLOW*** ⑤
			mix well
			measure tracer

Aflatoxin M1 / solid samples: ***Solid Sample***

- Recommended for milk powder

Procedure:

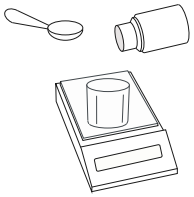
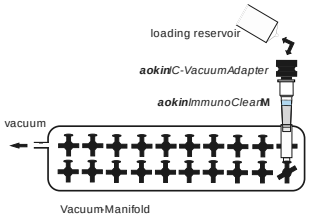
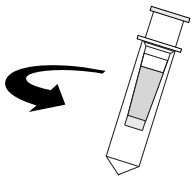
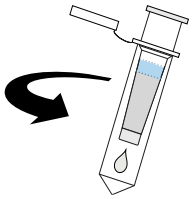
Extraction		**Weighing:** 8 g sample 80 mL <u>destilled</u> or <u>deionized</u> water
		(Centrifuge 15 minutes at 15.000 x g, use defatted layer) 60 ml skim milk sample
Purification		set up column
		50 ml Load skimmed milk sample, 1 drop / second (slow flow rate) 40 ml wash column with ***aokin****ICWash* **AFLA M1** Ⓦ
		1 min centrifuge at 1000 x g (waste tube)
		**Elution:** 400 µl ***aokin****ICElute* ***AFLA M1*** 3 min incubation with lid on 1 min centrifuge at 3000 x g, use eluate for measurement
Measurement	Mix and read	**Quantification** add into tube: 960 µl ***aokin*** *ReactionBuffer* ④ 100 µl sample 20 µl ***AFLA M1*** *Reagent* ***BLUE*** ⑤ mix well measure background 20 µl add ***AFLA M1*** *Tracer* ***YELLOW*** ⑤ mix well measure tracer

Aflatoxin M1 / solid samples:

- Recommended for galactose

Solvable Samples

Procedure:

Extraction		**Weighing:** 5 g sample 75 mL destilled or deionized water
Purification		set up column
		75 ml Load all of the sample, 1 drop / second (slow flow rate) 40 ml wash column with ***aokin****ICWash* **AFLA M1** Ⓦ
		1 min centrifuge at 1000 x g (waste tube)
		**Elution:** 400 µl ***aokin****ICElute* ***AFLA M1*** 3 min incubation with lid on 1 min centrifuge at 3000 x g, use eluate for measurement
Measurement	Mix and read	**Quantification** add into tube: 960 µl ***aokin*** *ReactionBuffer* ④ 100 µl sample 20 µl ***AFLA M1*** *Reagent* ***BLUE*** ⑤ mix well measure background 20 µl add ***AFLA M1*** *Tracer* ***YELLOW*** ⑤ mix well measure tracer