

Read this package insert carefully before use. For more detailed information, please refer to the corresponding manual. For analytical parameters see validation report.

## SAFIA Mycotoxin Kit

### Intended Use

The SAFIA rapid test kits are designed to detect regulated mycotoxins in food and feed, including ochratoxin A, fumonisins, deoxynivalenol, zearalenone, aflatoxins, and T2 toxin. The SAFIA Kit is intended to be used by trained personnel. The substance to be tested depends on the type of kit. Available Kits are:

- **Storage toxin kit:** OTA, AFL + Control  
(Order number: STO1L001)
- **Field toxin kit:** FUM, DON, ZEN, T-2 + Control  
(Order number: FIE2L003)
- **Screening kit:** OTA, AFL, FUM, DON, ZEN, T-2 + Control  
(Order number: SCR1L013)

### Principle of Assay

SAFIA (Suspension Array Fluorescence Immunoassay) is a multiplex rapid test using coded microparticles. Particles for different analytes (mycotoxins and control) are differentiated via intrinsic red fluorescence of the particles. It employs an indirect competitive immunoassay where mycotoxins on the particles compete with sample mycotoxins for binding to specific antibodies. The generated signal, measured by a flow cytometer, is inversely proportional to the mycotoxin concentration. SAFIA is a mix-and-read immunoassay, requiring no washing steps, and includes an internal control to ensure accurate results.

### Storage and Stability

The kit should be stored in a **refrigerator at 2 – 8 °C**. The kit must never be frozen in a freezer, e.g. at – 20 °C! Direct exposure to light should be avoided. No guarantee can be given after the expiry date. Individual reagents from the kit must not be interchanged with other reagents from other kits, even if the same batch number is printed on the kit.

### Kit Components

- Black 96-well microtiter plate: 1, ready to use
- Calibration standards: 8 x 0.5 mL, ready to use, labelled "Kal-1" to "Kal-8"
- Sample buffer: 1 x 15 mL, 10-fold concentrate
- Primary antibodies: 1 x 5 mL, ready to use, labelled "AK 1"
- Secondary antibodies: 2 x 5 mL, ready to use, labelled "AK 2"
- Particle stock solution: 1 x 45 µL, concentrate, in a vial with insert
- Particle buffer: 1 x 1.5 mL, ready to use
- Fixation solution: 1 x 10 mL, ready to use

### Hazard and Precaution Statements

The kit components Calibration Standards and Fixation solution contain small amounts of mycotoxins and should therefore be handled with care.

### FOR RESEARCH USE ONLY

The kits may contain substances that are hazardous to health. Please refer to the safety data sheets (SDS) for safety instructions and precautionary measures for the components contained.

### Additional Required Materials/Equipment

#### Devices

- Microtiter plate shaker
- Shaker for vessels
- Centrifuge
- Analytical balance
- Single and multichannel micropipettes
- CyFlow® Cube 6 flow cytometer with CyFlow® Robby autoloading station (for microtiter plate format)
- Clean, sealable glass jar (Approx. 200 mL)
- Tubes for sample weighing and extraction
- Sample tubes for Sysmex Cube 6 flow cytometer (Sysmex order number: 04-2000)

#### Additional reagents required

- Ethanol suitable for analyses. Dilute to 70 % (vol/vol) for analyses, depending on sample type.
- Distilled or deionized water.
- SAFIA PVPP adsorber: ready to use, optionally available (Order number: SPVA-007) for nuts and decolorization of red juices.
- SAFIA PA adsorber: ready to use, optionally available (Order number: SPAA-008) for herbs and spices.

### Instructions

#### Preliminaries

Bring all reagents to room temperature before starting. Shake the fixation solution gently to dissolve any precipitates, which might form during storage at 2 – 8 °C.

**Diluting Sample Buffer:** Mix 15 mL (one bottle) sample buffer 10x concentrate with 135 mL deionized water. Precipitate may form in concentrate at 2 – 8 °C but will dissolve upon dilution. Ensure complete dissolution, rinsing the bottle with diluted buffer if needed.

**Preparing Plate Layout:** Use SAFIA Score software to allocate samples and standards to the microtiter plate. **Ensure every plate includes at least an eight-point calibration curve with duplicate measurements.**

#### Preparing Particle Working Solution

**For Entire Plate:** Dilute the entire particle stock (approx. 50 µL) in particle buffer. Shake well for at least 20 seconds to ensure a homogeneous suspension.

**For Partial Plate:** Prepare 110 µL per strip by diluting particle stock solution 1:33 (e.g., 10 µL stock solution with 320 µL buffer). Shake well before use.

**Required Volumes per Well/Strip (8 wells):**

• Particle solution	10 µL/well	110 µL/strip
• AK 1:	25 µL/well	350 µL/strip
• AK 2:	50 µL/well	700 µL/strip
• Fixing solution:	50 µL/well	700 µL/strip
• DI water:	140 µL/well	1,400 µL/strip

**Sample Preparation****Solid (Grain) Food and Feed Samples (Dilution Factor 16)**

1. Weigh 5 g of sample material.
2. Add 20 mL of 70 % (vol/vol) ethanol and shake the samples for 15 min, e.g. in an overhead shaker.
3. Centrifuge the mixture for 5 min at 1000 g.
4. Take the supernatant and dilute 250 µL of the extract in 750 µL sample buffer. The dilution must be shaken briefly and then centrifuged at 12,000 g for 10 min.

**Herbs and Spices (Dilution Factor 32)**

1. Weigh 5 g of sample material.
2. Add 40 mL of 70 % (vol/vol) ethanol and shake the samples for 15 min, e.g. in an overhead shaker.
3. Centrifuge the mixture for 5 min at 1000 g
4. Take the supernatant and dilute 250 µL of the extract in 750 µL sample buffer. The dilution must be shaken briefly.
5. Now add 2 spatula tips SAFIA PA-Adsorber (approx. 15-25 mg) per 1mL of diluted sample and shake the sample for 15 min.
6. Centrifuge again for 10 min at 12,000 g.

**Nuts (Dilution Factor 16)**

1. Weigh 5 g of sample material.
2. Add 20 mL of 70 % (vol/vol) ethanol and shake the samples for 15 min, e.g. in an overhead shaker.
3. Centrifuge the mixture for 5 min at 1000 g
4. Take the supernatant and dilute 250 µL of the extract in 750 µL sample buffer. The dilution must be shaken briefly.
5. Now add 3 spatula tips SAFIA PVPP-Adsorber (approx. 15-25 mg) per 1mL of diluted sample and shake the sample for 15 min.
6. Centrifuge again for 10 min at 12,000 g.

**Fruit Juices (Dilution Factor 8)**

1. Weigh 5 g of sample material. Fruit juice concentrates must be diluted with deionized water according to the concentration factor.
2. Add 5 mL 100 % (vol/vol) ethanol and shake the samples briefly.
3. **Decolorize Red juices (high content of anthocyanins):** Transfer 1 mL of sample extract to a new vial and add 3 spatula tips SAFIA PVPP-Adsorber (approx. 15-25 mg) for decolorization and shake the sample for 15 min, e.g. in an overhead shaker. Afterwards, centrifuge the mixture for 5 min at 1000 g.
4. Take the supernatant and dilute 250 µL of the extract in 750 µL sample buffer. The dilution must be shaken briefly and then centrifuged at 12,000 g for 10 min.

Alternatively, a laboratory paper filter can be used instead of centrifugation. **Further protocols for specific matrices and applications, as well as updates to existing protocols, are available at [www.safia.tech](http://www.safia.tech). Please check regularly for updates.**

**Assay execution**

1. Add 25 µL of diluted sample or standard to each well. **Use a new pipette tip for every new standard or sample. Only use the provided plate.**
2. Sequentially add:
  - 10 µL particle working solution (shake prepared working solution for at least 20 seconds before use)
  - 25 µL primary antibody (AK 1)
  - 50 µL secondary antibody (AK 2)
3. Incubate the plate for 20 minutes, shaking with a microtiter plate shaker.
4. Add 50 µL fixing solution, shake for another 5 minutes
5. Add 140 µL DI water.
6. Start measurement in the flow cytometer within the next hour.

Use a multichannel pipette or stepper, with a new tip for each reagent/standard. Avoid long intervals and ensure continuous pipetting between strips. Do not touch the liquid in the wells with the pipette tips if reusing tips for multiple wells.

**Read-out and Data Analysis**

Before starting your measurement, check the device performance using the SAFIA Check. Ensure that the Sheath Fluid is filled and the Waste Container is empty. Open the configuration file Mycotoxins-SCR\_A\_Robby.cv85, go to Work, assign a Tray ID, and select the wells to be measured. Verify the following settings: Sample Source Autoloader, 0.5 µL/s flow rate, sample volume 10-20 µL, sample mixing off, sample-to-sample cleaning set to standard. Start the measurement using the Play button. After the run, your data will be converted in FCS Express and can then be analyzed with SAFIA Score.

**Disposals Procedure**

All reagents and materials must be disposed of properly and responsibly after use. Please observe the applicable national regulations for disposal and refer to the safety data sheets if necessary.

We recommend decontaminating glassware or other equipment that has come into contact with solutions containing toxins using either an alkaline rapid cleaner or a 10 % hypochlorite solution.

SAFIA Manual: [safia.tech/manual/](http://safia.tech/manual/)

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**Symbols**

	Reference number		Consult instruction for use
	Batch Code		Manufacturer
	Use-by-date		Temperature limit