l'screen AFLA

Enzyme immunoassay for the detection of aflatoxin (Cat.nr. HU0040019 / HU0040039)

l'screen **AFLA** is a kit prepared for a competitive immunoenzymatic assay for the quantitative analysis of aflatoxin.
The kit contains the procedure and the materials sufficient for 96 determinations ***(Cat.nr. HU0040019)*** or 48 determinations ***(Cat.nr. HU0040039)*** including standards.
For result evaluation a microtiter plate or a strip photometer is required (manual or automatic ELISA reader).

Type of samples that can be analyzed (matrices)
Cereals, paprika, chilli, soy, dried fruits and nuts samples.

Sample preparation
- Cereals, dried fruits and nuts samples: Grinding, extraction in methanol-water, filtration or centrifugation, dilution.
- Paprika, chilli: Grinding, extraction in methanol-water-hexane, filtration, column purification, evaporation, resuspension, dilution.

Assay time: 50 minutes (sample preparation not included).

Detection limit
- Maize, durum wheat, hazelnut, pistachio nut: 0,5 ppb
- Paprika/chilli, raisins,figs: 1,25 ppb.

Specificity	
Compound	**Cross-reactivity**
Aflatoxin B1	100%
Aflatoxin B2	29.2%
Aflatoxin G1	59.3%
Aflatoxin G2	6.3%
Aflatoxin M1	N.D*

* not detectable

1. TEST PRINCIPLE

The assay is performed in plastic microwells which have been coated with anti-rabbit antibodies. Aflatoxin standard solutions or samples, the enzyme conjugate and a specific antibody anti-aflatoxin are added to the microwells
During the incubation, free aflatoxin molecules and the enzyme conjugate compete for the anti-aflatoxin antibodies binding sites. The anti-aflatoxin antibodies are simultaneously bound to the solid phase. Any unbound substance is then removed in a washing step.
The bound enzyme activity is determined by adding a fixed amount of a chromogenic substrate. The enzyme converts the colorless chromogen into a blue product during the second incubation.
The addition of the stop reagent leads to a color change from blue to yellow. The absorbance is measured by a microplate reader at 450_{nm}. The color development is inversely proportional to aflatoxin concentration in the sample/standard

2. PROVIDED REAGENTS

Microtiter plate: coated with anti-rabbit antibodies.
As the strips are breakable, the wells can be used individually. For this purpose, it is sufficient to get out the wells from the frame and to break the joint.
Std Aflatoxin B_1: 6 plastic vials containing the aflatoxin in the following concentrations: 0 ng/ml; 0.05 ng/ml; 0.1 ng/ml; 0.2 ng/ml; 0.4 ng/ml; 0.8 ng/ml.
Anti aflatoxin antibody: 1 plastic bottle.
Enzyme conjugate: 1 plastic bottle.
Dilution buffer 1X:1 plastic bottle.
Washing-buffer 10X: 1 plastic bottle.
Developing solution:1 plastic bottle.
Stop solution: 1 glass bottle. White cap.

Component	Cat.nr. HU0040019 96 det.	Cat.nr. HU0040039 48 det.
Microtiter plate	96 wells (12 strip x 8 wells)	48 wells (6 strip x 8 wells)
Aflatoxin B_1 Std	6 vials x 1.5 ml	6 vials x 1.5 ml
Enzyme conjugate	14 ml	8 ml
Anti-Aflatoxin antibody	8 ml	5 ml
Dilution buffer 1x	50 ml	50 ml
Washing buffer 10x	50 ml	50 ml
Developing solution	24 ml	14 ml
Stop solution	8 ml	6 ml

3. MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled water
- MeOH (for all matrices).
- NaCl (for all matrices).
- Immunoaffinity columns (for paprika/chilli). *Contact technical assistance*
- n-hexane (for paprika/chilli).
- PBS (pH 7.2; for paprika/chilli).

Equipment:
- Balance.
- For grinding: grinder or blender (like "Osterizer").
- For extraction (optional): shaker
- Centrifuge (optional) or membrane filter 0.45 μm
- Filter paper (Whatman 1).
- Glassfiber filter (Whatmann 934-AH (for paprika/chilli).
- Evaporator.
- Vacuum manifold (like VacMaster)
- 20-200 µl, micropipettes, tips.
- 100-1000 µl, micropipettes, tips.
- 50-300 µl, multichannel micropipette, tips.
- Microplate plate, filter 450nm

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4. WARNING AND PRECAUTIONS FOR THE USERS

- The test is for *in vitro* diagnostic use only.
- Some reagents contain solutions that may be identified as dangerous substance by the Regulation (EC) *N° 1272/2008.* Please refer to *Material Safety Data Sheet* available on both the Eurofins Technologies and Eurofins Tecna (*tecna.eurofins-technologies.com)* web site.
- Handle the reagents with caution, avoiding contact with skin, eyes and mucous membranes.

5. HANDLING AND STORAGE INSTRUCTIONS

- Store the kit at +2/+8 °C and never freeze.
- **Bring all reagents to room temperature before use (at least 1 hour). ATTENTION: Do not unseal the microplate until it reaches the room temperature.**
- Reseal the unused strips of the microtiter plate in the bag together with the desiccant bag provided.
- Return all reagents to +2/+8 °C immediately after use.
- Do not use components after the expiration date.
- Do not intermix components from different kit lots.
- Do not use photocopies of the instruction booklet. Follow the original instruction booklet that is included with the kit.
- Do not change the assay procedure, in particular:
  - do not prolong the incubation times,
  - do not incubate the plate at temperatures higher than 25°C,
  - do not shake the plate during the incubations.
- Use accurate and precise micropipettes with suitable tips for dispensing.
- Once started, complete all the steps without interruption.
- The reproducibility of ELISA results depends largely upon the efficiency and uniformity of microwells washing; always keep to the described procedure.
- Use a single disposable tip for each standard solution and sample to avoid cross-contamination.
- Do not allow tips to contact the liquid already in the microwells.
- Avoid exposure to direct light during all incubations. It is recommended to cover the microtiter plate without using plate sealers.

6. SAMPLES PREPARATION

6.1 Cereals

1) Mix carefully the sample to be analysed in order to make it homogeneous.
2) Finely grind the sample.
3) Weigh 50 g of sample finely grinded
4) Add 5 g of NaCl.
5) Add 100 ml of MeOH 80%
6) Mix thoroughly for 3 minutes using a high-speed shaker or for 15 minutes using a low speed shaker.
7) Filtrate (Whatman 1) or centrifuge at 3500g for 5 minutes.
8) Mix 100 µl of supernatant or filtrate with 400 µl of dilution buffer.
9) Filtrate on a 0.45 µm filter or centrifuge the sample 3500g for 10 minutes or 8000g for 5 minutes
10) The dilution factor is 10.

6.2 Paprika and chilli samples

1) Mix carefully the sample to be analysed in order to make it homogeneous.
2) Finely grind the sample.
3) Weigh 5g of sample finely grinded
4) Add 0.5g of NaCl.
5) Add 25 ml of methanol 80%
6) Add 12.5ml of n-hexane.
7) Shake for 3 minutes at 350-400 rpm.
8) Filter on cellulose filter Whatman 1.
9) Rescue the lower methanolic phase.
10) To 2.8 ml of the methanolic phase add 17.2 ml of PBS 1X (pH 7.2).
11) Filter on glassfiber filter (Whatman 934-AH).
12) *Column preparation*: eliminate the storage solution from the column. **ATTENTION***:* Bring the immunoaffinity columns to room temperature before use.
13) Add 10 ml of the extract (2ml/min or gravity).
14) Wash the column with 10 ml of PBS1X.
15) Remove the PBS residue with vacuum.
16) Elute with methanol 100% (1 ml for 2 times)
17) Rescue completely the methanol from the column.
18) Evaporate the eluate at 40°C under a slow air or nitrogen stream.
19) Dissolve the residue in 1.4 ml of methanol 80%.
20) Dilute 100 µl of the eluate with 400 µl of dilution buffer.
21) The dilution factor is 25.

6.3 Nuts, soy

1) Mix carefully the sample to be analysed in order to make it homogeneous.
2) Finely grind the sample.
3) Weigh 50 g of sample finely grinded.
4) Add 5 g of NaCl.
5) Add 100 ml of a solution of MeOH 60%
6) Mix thoroughly for 3 minutes using a high-speed blender or for 15 minutes using a low speed shaker.
7) Filtrate the sample (Whatman 1) or centrifuge at 3500xg for 5 minutes and recover the supernatant /filtrate.
   ATTENTION*:* For pistachio centrifuged samples recover intermediate layer (do not take upper greenish layer).
8) Prepare a solution of 5% methanol in dilution buffer (example: 0.5 ml of 100% methanol + 9.5 ml of dilution buffer).
9) Dilute 100 µl of extract in 400 µl of 5% methanol buffer.
10) Centrifuge at 3500xg for 10 minutes or 8000xg for 5 minutes.
11) The dilution factor is 10.

6.4 Dried fruits

1) Mix carefully the sample to be analysed in order to make it homogeneous.
2) Finely grind the sample.
3) Weigh 5 g of sample finely grinded.
4) Add 0.5 g of NaCl.
5) Add 25 ml of MeOH 80%
6) Mix thoroughly for 3 minutes using a high-speed blender or for 15 minutes using a low speed shaker.
7) Filtrate the sample (Whatman 1) or centrifuge at 3500xg for 5 minutes; recover the supernatant /filtrate.
8) Dilute 100 µl of the eluate in 400 µl of dilution buffer.
9) The dilution factor is 25.

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7. WORKING SOLUTIONS PREPARATION

Std Aflatoxin B_1: ready to use.
Enzyme Conjugate: ready to use.
Anti- Aflatoxin antibody: ready to use.
Dilution buffer: ready to use.
Washing buffer: dilute the concentrate 1:10 (1+9) with distilled water. **ATTENTION**: In presence of crystals, bring the solution at room temperature and stir in order to solve them completely.
The diluted washing buffer is stable at room temperature for 24 hours and at +2/+8°C for two weeks.
Developing solution: ready to use; this solution is light sensitive: keep away from direct light.
Stop solution: ready to use. **ATTENTION**: It contains 1M sulphuric acid. Handle with care; in case of contact flush immediately with plenty of water.

8. ASSAY PROCEDURE

1) Predispose an assay layout, recording the standard and samples positions, taking into account that all have to be run in duplicate.
2) First incubation
   - Add 50 µl of each standard/ sample into the corresponding wells.
   - Using the multichannel pipet, add 100 µl of enzyme conjugate in each well.
   - Using the multichannel pipet, add 50 µl of antibody in each well.
   - Shake the plate gently with rotatory motion for few seconds.
   - Incubate 30 minutes at room temperature.
   - Do not prolong the first incubation time and do not use automatic shakers.
3) Washing
   - Pour the liquid out from the wells.
   - Fill completely all the wells with working wash solution using a squeeze bottle. Pour the liquid out from the wells.
   - Repeat the washing sequence four (4) times.
   - Remove the remaining droplets by tapping the microplate upside down vigorously against absorbent paper.

Do not allow the wells to dry out

4) Developing
   - Using the multichannel pipet, add 200 µl of developing solution to each well.
   - Mix thoroughly with rotatory motion for few seconds.
   - Incubate for 20 minutes at room temperature.
5) Using a multichannel pipet, add 50 µl of stop solution to each well and mix thoroughly with rotatory motion for few seconds.
6) Measure the absorbance at 450 nm.
7) Read within 60 minutes.

9. CALCULATION OF RESULTS

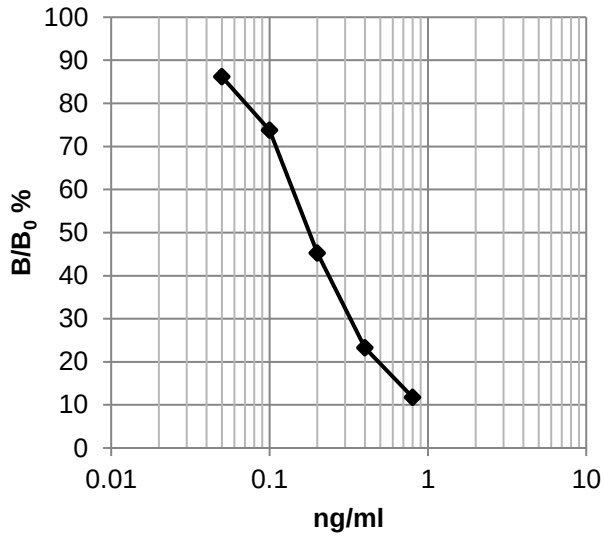
- Calculate the mean absorbance of each control, standard and sample.
- Divide the mean absorbance value of each standard and sample by the mean absorbance of the standard 0 and multiply by 100; the standard 0 is thus made equal to 100% and all the other absorbance values are expressed as percentages:

$$\frac{\text{Standard (or sample) absorbance}}{\text{Standard 0 } (B_0) \text{ absorbance}} \times 100 = \frac{B}{B_0}(\%)$$

- Enter the B/B_0 values calculated for each standard in a semi-logarithmic system of coordinates and draw the standard curve.
- Interpolate the B/B_0 value of each sample to the corresponding concentration from the calibration curve. Multiply this concentration for the dilution factor, as reported in chapter 6.

Please note: For results calculation, Excel spreadsheets are available on Eurofins Tecna website tecna.eurofins-technologies.com and can be downloaded directly from the bottom of the product page.

10. CALIBRATION CURVE EXAMPLE



11. EVALUATION OF RESULTS

After processing the results, it is necessary to verify the assay performance. The verification is performed by comparison of obtained data with those given in kit specifications (see chapter 12).
If the values are outside the specifications given, then the results of the test are not assured, therefore the aflatoxin concentration levels in the samples may not be valid.
In these cases it is advised to check the expiry date of the kit, the wavelength at which the reading was performed, as well as the procedure followed.
If operation errors are not identified as cause, contact our technical assistance.

WARNING: Kit replacement will only be possible in case of return. The kit must be stored in its integral version at +2/+8°C.

12. KIT SPECIFICATIONS

12.1 Assay specification

Description	Specifications
Mean B_0 absorbance	$\geq$ 0.7 OD_{450nm}
B/B_0 50 %	0.1-0.3 ng/ml
Std duplicates mean C.V.	$\leq$ 6 %

12.2 Assay performance

Matrix	Cut off ppb	LOQ ppb
Paprika/chilli	2.4	2.5
Maize	1.8	2
Durum wheat	$\leq$ 0.5	0.5
Hazelnut	$\leq$ 0.5	1
Pistachio nut	0.7	1
Raisins	$\leq$ 1.25	2
Figs	$\leq$ 1.25	2

13. LIABILITY

Samples evaluated as positive using the kit have to be re-tested with a confirmation method.
Eurofins Technologies Hungary shall not be liable for any damages to the customer caused by the improper use of the kit and for any action undertaken as a consequence of results.
Eurofins Technologies Hungary shall not be liable for the unsafe use of the kit out of the current European safety regulations.

14. LITERATURE

Lai J., Adrian D., Fragassi S., Gili M., Gramaglia M., Cazzola P.L., Decastelli L., Cooperazione Italia - Bielorussia: utilizzo e validazione di un metodo "Elisa" per la determinazione del contenuto di aflatossine totali in alimenti ad uso zootecnico. Biologi Italiani, XXXVIII-8, 64-67 (2008).
Decastelli L., Lai J., Gramaglia M., Monaco A., Nachtmann, C., Oldano F., Riffier M., Sezian A., Bandirola C. (2007). Aflatoxins occurrence in milk and feed in Northern Italy during 2004-2005. Food Control 18(10):1263-1266.

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