



A Rapid Test for Aflatoxin B1 Detection in maize and pistachios.

INCUBATION TEMPERATURE: 40°C

I. Introduction

Aflasensor is a **quantitative** rapid test that allows you to detect Aflatoxin B1 (AFB1) molecules in maize and pistachios samples. After an extraction procedure of the sample (section II), the test takes **10 minutes** at 40°C (using Heatsensor). The results are interpreted with an instrumental reading (Readsensor) for quantitative measurements. The LOQ of Aflasensor is **2 µg/kg** with a **quantification range** going from **2 to 500 µg/kg in Maize** and from **5 to 1000 µg/kg in pistachios**.

II. Extraction protocol

Aflasensor is a dipstick test devoted to the analysis of ground cereals and dried fruits. The extraction protocol is very simple and direct. It is described in Figure 1.

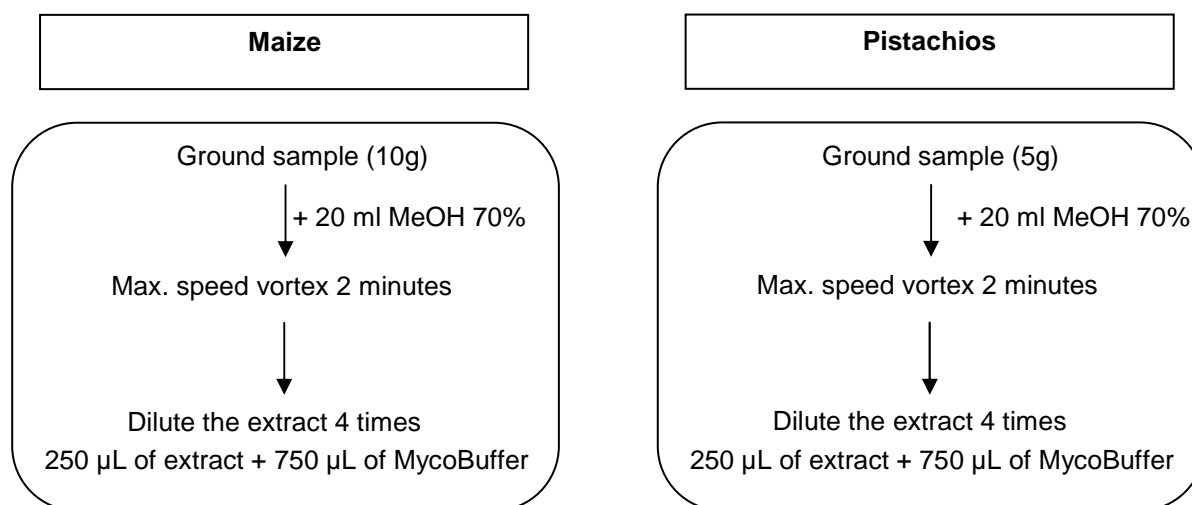


Figure 1 : Extraction protocol for analyzing AFB1 contamination in Maize and in pistachios.

Remarks :

- Grind your Maize or pistachios sample with a coffee grinder or something comparable so that the particles size is less than 20 Mesh.
- Falcon tubes of a 50 ml capacity with self-standing bottom must be used for the extraction experiment.
- Ultra pure, MilliQ or desionized water can be used for the extraction.
- Vortex should operate at the highest speed.
- After extraction, let stand your extract during 2 minutes until you can remove 250 µL avoiding particulates or centrifuge it.
- Mix gently your diluted extract several times before use.
- Use always fresh extracts and diluted extracts directly after preparation.

III. Summary of the dipstick test protocol

- Add **200 µL** of the diluted maize or pistachios extract into one reagent microwell and mix **10 times** to homogenize;
- Incubate for **1 min at 40°C** ;
- Dip one dipstick into each microwell;
- Incubate for **9 min at 40°C** ;
- Directly measure the colour intensities with readsensor.

IV. Reaction Mechanism

Aflasensor is a competitive test involving specific antibodies with high affinity for AFB1 molecules. The test requires the use of two components. The first component is a microwell containing predetermined amount of antibody linked to gold particles. The second is a dipstick made up of a set of membranes with specific capture lines. For a valid test, the upper red control line has to be visible after the test. When the reagent from the microwell is re-suspended with a cereal or dried fruits extract, specific antibodies will bind the analytes if present. When the dipstick is dipped into the sample, the liquid starts running vertically on the dipstick and passes through capture zones. When the sample is free of AFB1, a colour development occurs at the “test” line, indicating its absence in the cereal or dried fruits sample. On the opposite, the presence of AFB1 in the sample will not cause the appearance of the coloured signal at the “test” capture line.

V. Composition of kits

Aflasensor Kits contain the reagents needed to perform 24 measurements.

- 3 pots each with 1 strip of 8 reagent microwells and 8 dipsticks;
- One flask containing 30 ml of MycoBuffer;
- One instruction sheet with the summary of protocol.

VI. Additional material needed

- 1 Heatsensor (40°C incubation, refer to the used Heatsensor manual);
- 1 Readsensor (refer to the Readsensor manual);
- 1 vortex (used at its highest speed);
- Falcon tubes of 50 ml with a self-standing bottom;
- Ultra Pure Water;
- Methanol (CAS: 67-56-1);
- Eppendorfs for the extracts dilution with the MycoBuffer;
- Micropipettes.

VII. General Remarks

1. The Readsensor must be switched on before the analysis (see Readsensor manual);
2. At reception, store the kit in a dry place and at a low temperature between 2°C and 8°C. Before opening, let the plastic pots reach room temperature and avoid exposure of the product to moisture and light;
3. The best temperature to perform the test is **40°C ± 1°C** . Use the “Heatsensor” (or alternatively a water bath). Any other type of incubator is not appropriate to perform this dipstick assay. (Refer to the used Heatsensor manual for setting at right temperature and timing);
4. After the dipstick incubation, read the result within a 2-minutes time frame. Do not attempt to interpret the result after 2 minutes;
5. When drying, the colour intensities of the lines will become sharper;
6. When a positive result is recorded, the test result should be confirmed;
7. Empty one pot before opening another pot.

VIII. Directions for use

This procedure is described to easily run one single sample or a set of many samples. In that case, try to perform the test in cascade and avoid any delays when mixing reagent and cereal or dried fruits extracts but also when adding and removing dipsticks. Make sure you have the same incubation time and temperature for each sample. You shouldn't test more than 6 samples at one time and, if there are more than 3 samples, you should use a multipipette. With more than 6 samples we recommend to share series of maximum 6 samples.

1. Choose a clean and dry place to perform the test and wash and dry your hands before starting;
2. Connect the Heatsensor and wait until the temperature has stabilised at 40°C.
3. Before opening the reagents, take the kit out of the fridge and wait until the temperature of the reagents reaches the ambient temperature. Meanwhile, read the directions for use attentively;
 - *There are two main components used for the test which are: dipsticks and the freeze-dried reagents in microwells. Both are stored in the white plastic pots.*
4. Determine how many samples are to be tested and write on each sample an identification number;
 - *The maize and pistachios samples must be milled in flour before starting the extraction experiments (particles size < 20 mesh). After extraction, directly dilute 4 times the extract in the MycoBuffer, mix gently to homogenize.*
5. Open one plastic pot and take out as many microwells and dipsticks as there are samples to be tested;
 - *To open a pot of dipsticks, take off the safety ring by pressing it down the pot, take off the ring and get the stopper off the pot with your thumb;*
 - *The pot with dipsticks should always be well closed after reagents have been taken out;*
 - *A pot with dipsticks should be emptied before another is opened;*
 - *Be careful, if you do not intend to use all the 8 microwells, leave the set of 8 caps on the unused ones and do not tear off the strip of the eight caps but leave it on the microwells that will not be used. Do not try to separate individual caps and put them immediately back into the white pot without damaging the dipsticks, close and make sure it is tightly sealed.*
 - *Write down on each dipstick intended to be used the number that matches the one of the samples*
6. Place the microwell(s) in the heating block which shows 40°C;
7. Place a new tip on the micropipette and rapidly transfer 200 µL of diluted extracts into each of the microwells, mix 10 times to homogenize;
8. **Warning: when reagents and the extract are in contact, the reaction begins. Quickly mix AND IMMEDIATELY push the START (RUN)* button. The 1-minute countdown starts;**
9. After the 1-minute incubation, insert the dipsticks in the corresponding microwells and **incubate 9 minutes** again at 40°C;
10. When the 9 minutes are over, i.e. after the sound-signal, press START (STOP)* to stop the ringing tone and take the dipsticks out of the microwells to lay them down on a sheet of paper, remove the filter pad and directly interpret the result with Readsensor;
11. If you are not planning to perform any other test within the day with Aflasensor, put everything back into the box and store it in a fridge at a temperature ranging from 2 to 8°C.

**(Refer to the Heatsensor manual - For DUO Heatsensor users, step 8 and 9 is modified)*

IX. Results interpretation

Interpret the results with Readsensior. Remove the filter pad out of the dipstick directly after the end of the incubation (described in paragraph VIII) and interpret the results as soon as possible **no later than 2 minutes** after the end of analysis.

- The Readsensior must be switched on before the analysis with the appropriated reading method (KIT062_Maize, _pistachios) [#]. Each dipsticks batch has indeed its own calibration curves.
- As soon as the dipstick migration is ended, remove the sample pad and insert the dipsticks consecutively in the Readsensior and press the central button [#].
- On the basis of the test/control line ratio, the reader will convert this value to a concentration measured in “part per billion” (ppb) present in your maize or pistachios sample. Three possibilities can be encountered :
 - If your sample contains less than 2 or 5 ppb of AFB1 respectively for maize and pistachios, then the reader will display “<2ppb” or “<5ppb”.
 - If your sample contains a AFB1 concentration between 2 and 60 ppb or between 5 and 200 ppb respectively for maize and pistachios, then the reader will accurately quantify this contamination.
 - If your sample contains more than 60 ppb of AFB1 in maize or more than 200 ppb in pistachios, then the reader will declare your sample “out range” and “DILUTE 10x” for maize (“DILUTE 20x” for pistachios) as result. If you still have any interest to quantify the AFB1 content, therefore, you should dilute 10 times more your maize extract (or 20 times for pistachios extract) with the MycoBuffer and do the analysis again. At the end, do not forget to multiply the measured concentration by a factor of 10 (or 20 for pistachios). This procedure is valuable for contaminated samples until 500 ppb of AFB1 for maize and 1000 ppb for pistachios. Beyond these levels, the quantification becomes more tedious.

[#](See the Readsensior manual for more details)

X. Sensitivity

The use of Readsensior allows for an accurate quantification of the AFB1 contamination in maize and in pistachios in a range of 2 to 500 ppb in maize and 5 to 1000 ppb in pistachios. According to its cross-reactivity profile (Table 1), Aflasensior dipstick tests can be considered as specific to Aflatoxin B1.

Toxin	Cross-Reactivity (%)
AFB1	100
AFG1	8
AFB2	4
AFG2	1

Table 1 : Cross-reactivity profile of Aflasensior dipsticks towards Aflatoxins.