AFLATOXIN B_1 FLOW-THROUGH RAPID TEST

A Flow-Through enzyme immunoassay
for the detection of Aflatoxin B_1

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BRIEF INFORMATION

The Aflatoxin B_1 (AFB_1) Flow-Through Rapid Test is a competitive sequential enzyme immunoassay for the screening of food samples (oats, barley, rye, rice, wheat, millet, maize, buckweat, legumes, tree nuts, seeds, pine nuts, spices) for the presence of AFB_1. The test is based on antibodies against AFB_1. With this kit 10 analyses can be performed. The kit contains all the reagents required, to perform the test.

1. INTRODUCTION

Aflatoxins are extremely toxic compounds produced by the moulds *Aspergillus flavus, A. paraciticus* and *A. nomius* [1]. Aflatoxins cause cancer, mainly of the liver but also of the gut, lungs and breasts. These moulds mainly occur on food, feed and their ingredients derived from tropical and sub-tropical areas. The most pronounced contamination has been encountered in cereals, rice, maize, soy, tree nuts, spices and peanuts [2].
Maximum tolerance (MLs) for aflatoxins are legally established in Europe. Depending on the products used for animal feed or direct human consumption the MLs vary from 2 to 50 µg/kg (ppb) [3].
In the Aflatoxin B_1-kit of EuroProxima an antiserum is used that cross-reacts with the aflatoxins B_1 and to a lesser extend with the aflatoxins B_2, G_1 and G_2.

2. PRINCIPLE OF THE AFLATOXIN B_1 ASSAY

The membrane-based assay kit consists of 10 devices each pre-coated with rabbit antibodies to mouse IgG. Specific antibodies (mouse anti-AFB_1), sample, and enzyme labelled AFB_1 (enzyme conjugate) are added sequentially to the pre-coated membrane contained in the device. The specific antibodies are bound by the immobilised anti-mouse antibodies. On addition of the sample, AFB_1 (in the sample extract) then binds to the mouse anti-AFB_1 antibodies. After the sample extract has completely adsorbed through the membrane the enzyme labelled AFB_1 binds to free mouse anti-AFB_1 antibodies.

The unbound conjugate (enzyme labelled AFB_1) is removed by a washing step. A chromogen substrate (tetramethylbenzidine, TMB) is then added. Bound enzyme transforms the chromogen substrate into a blue coloured product and this appears as a line. The results are visually interpreted within 5 minutes. After the addition of the substrate.

10. LITERATURE

1. J.E. Smith, C.W. Lewis and J.G. Anderson. Mycotoxins in human nutrition and health. EU Directorate-General XII, Science, Research and Development, 1990.

2. J.L. Richard, G.A. Bennett, P.F. Ross and P.E. Nelson. Analysis of naturally occurring mycotoxins in feedstuffs and food. J. Anim. Sci. 71, 2563-2574, 1993.

3. Council Directive 1999/29EC of 22 April 1999 on the undesirable substances and products in animal nutrition. Off. J. European Commun. L115 (1999) 32-46.

   Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. Off. J. European Commun. L77 (2001) 1-13.

   Commission Regulation (EC) No 472/2002 of 12 March 2002 amending Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs.

11. ORDERING INFORMATION

For ordering the AFB_1 kit, please use cat. code 5127AFB01.
For ordering the Amino SPE Columns please use cat. code 8015NH2Column.
For ordering sample cups please use cat. Code 8015ET10.

EuroProxima B.V.
Beijerinckweg 18
NL-6827 BN Arnhem
The Netherlands

TEL: + 31 26 3630364
FAX: + 31 26 3645111
E-mail: info@europroxima.com
Internet: http://www.europroxima.com

3. HANDLING AND STORAGE

- Store the kit at + 2°C to + 8°C in a dark place.

- After the expiry date (see kit label) has passed, it is no longer possible to give a quality guarantee.

- Store the remaining devices in the resealable zip lock bag and refrigerate. Before opening the kit, allow it to reach ambient temperature.

- Any direct action of light on the chromogen solution should be avoided.

If the following phenomena are observed, this may indicate a degeneration of the reagents:
- A blue colouring of the chromogen solution before using it on the membrane.

4. KIT CONTENTS

Kit contents are sufficient to carry out at least 10 analyses and everything required for the assay is included except for the sample cups (Cat. Code 8015ET10). Certain matrices also require, additional clean-up columns (Cat. code 8015NH2Column).

Kit Contents:

- 2x 5 membrane devices

- 10 filters

- 10 syringes

- 3 bottles Reagent A (extraction solution)

- 1 vial Reagent B (dilution buffer)

- 1 vial Reagent C (antibody solution, yellow cap)

- 1 vial Reagent D (enzyme conjugate, green cap)

- 1 vial Reagent E (washing buffer, white cap)

- 1 vial Reagent F (TMB substrate, blue cap)

5. SAFETY PRECAUTIONS

- Aflatoxins are carcinogenic and toxic compounds. Avoid contact with mouth and skin. Be aware the aflatoxins are not swallowed.
- Any material contaminated with aflatoxins should be destroyed or decontaminated.
- Avoid contact of all biological materials with skin and mucous membranes.
- Do not pipette by mouth.
- Do not eat, drink, smoke, store or prepare foods, or apply cosmetics within the designated work area.
- TMB is toxic by inhalation, in contact with skin and if swallowed; observe care when handling the substrate.
- Do not use components past expiration date and do not intermix components from different serial lots.
- Optimal results will be obtained by strict adherence to this protocol.

6. SAMPLE TREATMENT

Approximately 50 g of sample is ground.

(i) Sample extraction

(a) For the determination of 2 ppb:

- An amount of 5 g of ground sample is extracted with 15 ml of Reagent A.

(b) For the determination of 4 ppb:

- An amount of 2.5 g of ground sample is extracted with 15 ml of Reagent A.

(ii) Shake by hand at room temperature for 3 minutes and leave the sample to settle and to obtain clean supernatant.

(iii) Sample dilution step

For cereals, nuts and coated nuts:

Draw 1,4 ml of dilution buffer (Reagent B) with a syringe and draw 1 ml of supernatant to the 2.4 ml mark and mix gently, to obtain a 33 % of methanol concentration. The sample is ready for use. Fix the filter to the syringe.

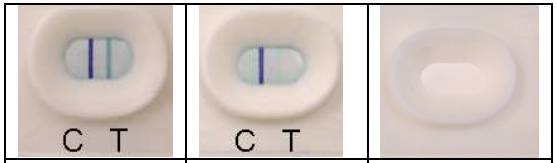
7. PREPARATION OF REAGENTS

Before starting the test, the reagents should be brought up to ambient temperature. Any reagent not used should be put back into storage immediately at + 2℃ to + 8℃.
All the reagents are provided ready to use.

8. ASSAY PROCEDURE

1. Add 2 drops of Reagent C onto the middle of the membrane. Allow liquid to flow-through completely.
2. Add 20 drops of sample extract with the syringe. Allow liquid to flow-through completely.
3. Add 2 drops of Reagent D. Allow liquid to flow-through completely.
4. Wash membrane with 1 drop of Reagent E. Allow liquid to flow-through completely.
5. Rinse membrane with 3 drops of Reagent E. Allow liquid to flow-through completely.
6. Add 5 drops of Reagent F and observe colour development. An optimal interpretation of results is achieved 5 to 6 minutes after application

9. INTERPRETATION OF RESULTS



Negative	Positive	Invalid (test again)

If the sample is negative for aflatoxins then two blue coloured lines will appear (C + T).
If the sample is positive for aflatoxins then only one blue coloured line will appear (C).
The test is invalid when no blue coloured line appears. The sample should be retested.