BioSystems

COD 14200 10 tests
Reagents for the determination of aflatoxin B1 in food samples
Only for *in vitro* use in the laboratory

AFLATOXIN B1 rapid test

ENZYME IMMUNOASSAY ON NITROCELLULOSE

GENERAL INFORMATION

Aflatoxins are a group of extremely toxic compounds produced by the moulds *Aspergillus flavus*, *A. paraciticus* and *A. nomius*[1]. These moulds may occur on food and feed obtained from tropical and sub-tropical areas. Aflatoxin contamination has been mainly found in cereals, rice, maize, soy, tree nuts and peanuts[2]. Aflatoxins cause cancer, mainly of the liver but also of the gut, lungs and breast.

Maximum tolerance limits (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)[3-5].

PRINCIPLE OF THE METHOD

The Aflatoxin B1 Rapid Test is a competitive enzyme immunoassay on nitrocellulose for the screening of aflatoxin B1 in food samples (oats, barley, rye, rice, wheat, millet, maize, buckweat, legumes, tree nuts, seeds, pine nuts, spices).

Rabbit antibodies to mouse IgG are immobilised in the test line (T) of the nitrocellulose membrane. A mouse anti-aflatoxin B1, sample, and enzyme labelled aflatoxin B1 are added sequentially. The mouse antibody binds to the immobilized rabbit antibody. Aflatoxin B1 in the sample competes with the conjugate to bind to the specific mouse antibody. The unbound conjugate is removed by a washing step. A chromogen substrate (tetramethylbenzidine) is then added. Bound enzyme transforms the chromogen substrate into a blue coloured product appearing as a color band.

CONTENTS AND COMPOSITION

Devices. 2 x 5 cassettes.

Reagent A. 3 bottles. Extraction solution.

Reagent B. 1 vial. Dilution buffer.

Reagent C. 1 vial. Antibody solution, yellow cap.

Reagent D. 1 vial. Enzyme conjugate, green cap.

Reagent E. 1 vial. Washing buffer, white cap.

Reagent F. 1 vial. Tetramethylbenzidine substrate, blue cap.

Filters and Syringes. 10 units each.

STORAGE AND STABILITY

Store at 2-8 ºC. Each component is stable until the expiry date marked in the label.

Liquid components are stable once opened until the expiry date marked in the label if they are stored at the recommended temperature, well closed and care is taken to prevent contamination during their use.

Indications of deterioration:

- Reagents: Blue color of the substrate (Reagent F).
- Devices (cassettes): rips on the sealing bag, presence of lines or spots in the membrane before performing the assay.

REAGENT PREPARATION

All the reagents are provided ready to use.

PRECAUTIONS

- Aflatoxins are carcinogenic compounds. Avoid contact with mouth and skin. Be aware the aflatoxins are not inhaled. Any material contaminated with aflatoxins should be destroyed or decontaminated by addition of sodium hypochlorite solution (10% v/v).
- 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.

SAMPLE TREATMENT

A homogeneous sample has to be obtained from a representative part of the compound.

1. Sample (50-100 g) is ground and pulverised into a fine homogeneous compound.
2. Sample extraction:
   - For the **detection of 2 ppb**: 5 g of ground sample is extracted with 15 mL of Reagent A.
   - For the **detection of 4 ppb**: 2.5 g of ground sample is extracted with 15 mL of Reagent A.
3. Shake by hand at room temperature for 3 minutes and leave the sample to settle and to obtain clean supernatant.
4. **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. Mix gently. Fix the filter to the syringe.

   For other samples: Draw approximately 1.5 mL of the supernatant with a syringe. Fix the filter to the syringe.

PROCEDURE

Allow all the reagents and devices warm up to room temperature.

1. Open the Device package and take out the required amount of cassettes. Place the device on a flat surface. Store the unused cassettes into the sealing bag and reseal it, keeping the desiccant inside.
2. Add 2 drops of Reagent E onto the middle of the well. Allow liquid to flow-through completely.
3. Place 2 drops of Reagent C onto the middle of the well. Allow liquid to flow-through completely.
4. Add 20 drops of sample extract with the syringe. Allow liquid to flow-through completely.
5. Add 2 drops of Reagent D. Allow liquid to flow-through completely.
6. Wash membrane with 1 drop of Reagent E. Allow liquid to flow-through completely.
7. Rinse membrane with 3 drops of Reagent E. Allow liquid to flow-through completely.
8. Add 5 drops of Reagent F and observe color development. An optimal interpretation of results is achieved 5 to 6 minutes after application.

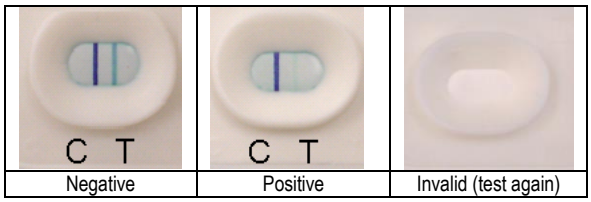
READING

Examine the presence of color bands inside the well of the cassette.

Negative result. Two color bands appear: one in the side "T" and another in the side "C" of the well.

Positive result. A color band appears only in the side "C" of the well.

Invalid result. Absence of color bands. Retest the sample using a new cassette.



Negative	Positive	Invalid (test again)

ASSAY CHARACTERISTICS

- Cut-off: 2-4 ppb.
- Specificity: The antiserum used cross-reacts with the aflatoxins B1 and to a lesser extend with the aflatoxins B2, G1 and G2.

BIBLIOGRAPHY

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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.
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