

AFLACARD TOTAL

Product Code: P38

Qualitative screening test for the detection of aflatoxins B1, B2, G1 and G2.
For in vitro use only.

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RHÔNE LTD

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Test Principle

The kit is based on monoclonal antibody technology which has the advantage of being highly specific and sensitive while the test format is rapid and simple to perform. The screening procedure is intended to serve as an indicator of the presence of toxins at various screening levels according to international legislation.

The toxins are extracted from the sample, filtered and passed through a clean-up column before being diluted and applied to the card. The conjugate is applied to the membrane and unbound conjugate is then removed by washing. A colourless substrate is added and the card is incubated for five minutes. Finally a stop solution is applied to the membrane. A purple spot must appear at the control site to indicate that the test is valid. A purple spot at the sample site shows that the contamination is less than the cut off value of the card. No colour at the sample site indicates contamination at a higher level than the cut off of the card.

The assay takes approximately 10 minutes to perform.

Kit Components

- 10 Cards (20 Determinations)
- 20 Clean-Up Columns
- 20 Filtrate Collection Tubes
- 20 Graduated Dilution Tubes
- 20 x 3 ml of Sample Diluent Buffer
- 2.5 ml of Ready-to-use Conjugate (red label)
- 4 ml of Wash Buffer (green label)
- 4 ml of Substrate (blue label)
- 4 ml of Stop Solution (yellow label)

Reagents Not Provided

- Distilled / Deionised Water (suitable for use with HPLC, e.g. MilliQ)
- Methanol

Accessory Products

- Whatman No. 113 or No. 4 Filter Paper

Recommended Methods and Application Notes

Methods are available for all matrices covered by legislation as well as additional commodities. Deviation from the methods described in our Instructions For Use and Application Notes may not result in optimum results. Please contact your local R-Biopharm distributor for further information.

Hazards

Mycotoxins are very hazardous substances. Only laboratories equipped to handle toxic materials and solvents should perform analyses. Suitable protective clothing, including gloves, safety glasses and lab coats should be worn throughout the analysis.

Flammable solvents should be stored in an explosion-proof cabinet. Use a chemical hood and protective equipment as applicable.

Contact your local R-Biopharm distributor for a Material Safety Data Sheet for further information if required.

Decontamination

Prior to disposal, excess standard solutions should be treated with at least one-tenth their volume of 5 % sodium hypochlorite. Labware and contaminated waste should be immersed in 5 % sodium hypochlorite solution for 30 minutes followed by the addition of 5 % acetone for 30 minutes. Flush with copious amounts of water before disposal. After decontamination labware should be thoroughly washed. Incinerate waste if regulations permit.

Storage & Shelf Life

The cards have an expiry of 12 months from date of manufacture if stored at 2 - 8 °C. Do not freeze.

Sampling

A representative sample should be obtained by following one of the officially recognised sampling procedures. It is recommended that a minimum of 1 kg of representative sample is finely ground and a portion (10 - 50 g dependent on method used) of this is removed and extracted.

Sample Preparation

• Cereal and Nuts

This method has been tested on a number of commodities including wheat, barley, maize, pistachio nuts, peanuts, almonds, Brazil nuts, hazelnuts and macadamia nuts.

1. Weigh 50 g of ground sample into a 1 litre capacity, solvent resistant blender jar.
2. Add 100 ml of 80 % methanol and blend at high speed for 2 minutes.
3. Filter the sample through Whatman No. 113 or No. 4 filter paper, or centrifuge at 4,000 rpm for 10 minutes.
4. Pass 5 ml of the filtrate through the solid phase clean-up column by applying pressure with the plunger and collect the cleaned-up filtrate in a filtrate collection tube. If the cleaned-up filtrate is clear it is ready for analysis. If it is cloudy it should be passed through the same clean-up column again before analysis.
5. Dependent on the screening level required add the appropriate volume of filtrate to a dilution tube containing the correct volume of 80 % methanol according to the table below.

Screening Level	Volume of Filtrate	Volume of 80 % Methanol
2 ppb	1 ml	0 ml
4 ppb	1 ml	1 ml
5 ppb	1 ml	1.5 ml
8 ppb	1 ml	3 ml
10 ppb	1 ml	4 ml
12 ppb	1 ml	5 ml
15 ppb	1 ml	6.5 ml
20 ppb	1 ml	9 ml
30 ppb	0.5 ml	7 ml

6. Add 1 ml of diluted filtrate to one of the vials containing 3 ml of sample diluent buffer.
7. The cleaned up solution is now ready for analysis. Please refer to Method section.

Sample Preparation

• Spices

This method has been tested on a number of spices including paprika, black pepper, white pepper, nutmeg and chilli powder.

Note: there is a specific application note available for turmeric.

1. Weigh 50 g of ground sample into a 1 litre capacity, solvent resistant blender jar.
2. Add 100 ml of 80 % methanol and blend at high speed for 2 minutes.
3. Filter the extract through a Whatman No. 113 or No. 4 filter paper, or centrifuge at 4,000 rpm for 10 minutes.
4. Dependent on the screening level required add the appropriate volume of filtrate to a dilution tube containing the correct volume of 80 % methanol according to the table below.

Screening Level	Volume of Filtrate	Volume of 80 % Methanol
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8 ppb	1 ml	3 ml
10 ppb	1 ml	4 ml
12 ppb	1 ml	5 ml
15 ppb	1 ml	6.5 ml
20 ppb	1 ml	9 ml
30 ppb	0.5 ml	7 ml

5. Add 1 ml of the diluted filtrate to one of the vials containing 3 ml of sample diluent buffer.
6. Pass the contents of the vial through the solid phase clean-up column by applying pressure with the plunger and collect the cleaned-up filtrate in a filtrate collection tube. If the cleaned-up filtrate is clear it is ready for analysis. If it is cloudy it should be passed through the same clean-up column again before analysis.
7. The cleaned up solution is now ready for analysis. Please refer to Method section.

Method

1. Remove the kit from the refrigerator and leave at room temperature for a minimum of 30 minutes before using the test.
2. Apply 500 µl of cleaned sample onto the port and let it pass through the membrane. This should take no more than 5 minutes.
3. Once the sample has passed through the membrane apply 100 µl of ready-to-use conjugate (red label) and let it pass through the membrane.
4. Once the conjugate has passed through the membrane apply 100 µl of wash buffer (green label). Allow the wash buffer to pass through the membrane and wipe around the port with a paper tissue.
5. Apply 100 µl of substrate (blue label) to the membrane and allow the colour to develop for 5 minutes (start the timer when the substrate is added).
6. After the required incubation apply 100 µl of stop solution (yellow label) and read results immediately after the stop solution has passed through the membrane.

Reading the Test Results

	Negative Result	The sample should be considered negative (below the cut-off point) when the sample and the control spot both have a clearly visible colour development.
	Positive Result	The sample should be considered positive (above the cut-off point) when the sample spot fails to develop a detectable colour.

The control spot must develop a clearly visible purple colour in order to have a valid test result. The colour in the sample and the control spot does not need to be of the same intensity. If doubt arises regarding the presence of a spot it is advised to hold the card at arm's length (against a dark background). If the spot can be clearly seen then it can be considered as being present.

Notes

- The unused card has two light blue coloured spots on each port which will disappear during the course of the assay. Ensure these are visible before starting the analysis. Note: these may be faint in appearance.
- Remember: It is possible to analyse two samples on one card, one sample per port. Each port has its own internal control.
- The second port must be used within 1 week of the first port. Care should be taken to ensure that the seal on the second port remains in place until it is required for use. It is advised to peel back the label from the first port and cut, keeping the label securely in place over the second port.
- Do not use reagents from one batch number in conjunction with reagents from a different batch. Do not substitute reagents from other manufacturers.
- Always ensure that there are no air bubbles in the substrate drops.
- Use of incubation times other than those specified may give inaccurate results.
- Always allow reagents to be completely absorbed before adding the next reagent.

Quality

RBR products are developed, manufactured, tested and dispatched under an ISO 9001 registered Quality Management System, guaranteeing a consistent product, which always meets our performance specifications. Our products have been used in many collaborative studies to develop standard European and International Methods and are widely used by key institutions, food companies and government laboratories. Customer references for RBR products are available on request.

Technical Support

RBR understand that from time to time users of our products may need assistance or advice. Therefore, we are pleased to offer the following services to our customers:

- Analysis of problem samples.
- Application notes for difficult samples.
- References from the RBR library.
- Installation and support of the KOBRA® CELL.
- Advice on detection parameters.
- Advice on preparation and handling of standards.
- Updates on legislation, sampling and other news by e-mail.
- Provision of spiked samples.

Please contact your local R-Biopharm distributor for further information.

Warranty

R-Biopharm Rhône Ltd makes no warranty of any kind, express or implied, except that all products made by R-Biopharm Rhône Ltd are made with materials of suitable quality. If any materials are defective, R-Biopharm Rhône Ltd will provide a replacement product. The user assumes all risk and liability resulting from the use of R-Biopharm Rhône Ltd products and procedures. R-Biopharm Rhône Ltd shall not be liable for any damages, including special or consequential damages, loss or expense arising directly or indirectly from the use of R-Biopharm Rhône Ltd products or procedures.

