OCHRACARD

Product Code: P48

Qualitative screening test for the detection of ochratoxin A.
For *in vitro* use only.

P48/V12/29.07.15

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R-BIOPHARM
RHÔNE LTD

Contents

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 toxin is extracted from the sample, filtered and passed through an immunoaffinity 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 30 minutes to perform.

Kit Components

- 10 Cards (20 Determinations)
- 20 Immunoaffinity Columns
- 10 PBS Tablets (Phosphate Buffered Saline)
- 20 Sample Collection Tubes
- 20 Filtrate Dilution Tubes
- 2 x 22 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
- Immunoaffinity Column Rack (CR1)*
- Immunoaffinity Column Accessory Pack (AP01)*

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

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 10 months from date of manufacture if stored at 2 - 8 °C. Do not freeze.

Ensure that the column has not dried out and contains buffer above the gel. It is important to note that the antibody included in the immunoaffinity column can be denatured by extreme temperature or pH change.

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.

Column Preparation

Immunoaffinity columns should be at ambient temperature before use. Remove the cap from the top of the column and discard. Firmly attach the column to a glass syringe barrel using an adapter and place in an immunoaffinity column rack or clamp stand.

Backflushing

Backflushing is carried out to increase the time the solvent is in contact with the antibody within the gel suspension ensuring that all of the toxin is eluted. Backflush by gently raising and lowering the syringe plunger during passage of the solvent through the column. This process will reverse the direction of flow of the eluant. This should be repeated 3 times.

Preparation of 0.01 M Phosphate Buffered Saline (PBS)

1. Dissolve 1 PBS tablet in 100 ml of water.

Sample Preparation

• Cereal

This method has been tested on a number of cereals including wheat, barley and maize.

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. Dilute 9 ml of filtrate with 18 ml of phosphate buffered saline (PBS).
5. Dependent on the screening level required pass the appropriate volume of diluted filtrate through the immunoaffinity column according to the table below.

Screening Level	Volume of Diluted Filtrate Applied to Column
3 ppb	25 ml
4 ppb	20 ml
5 ppb	15 ml
10 ppb	7.5 ml

6. Pass the required volume of diluted filtrate through the immunoaffinity column at a flow rate of 2 ml per minute (or the sample can be allowed to pass through the column by gravity if preferred). A slow, steady flow rate is essential for the capture of the toxin by the antibody.
7. Wash the column by passing 10 ml of PBS through at a flow rate of approximately 5 ml per minute. Pass air though the column to remove residual liquid.
8. Elute the toxin from the column at a rate of 1 drop per second using 1 ml of 100 % methanol and collect in an amber glass vial. Backflushing is recommended. Please refer to the Backflushing section for further information.
9. Following elution pass 2 ml of sample diluent buffer through the column and collect in the same vial to give a 3 ml total volume.
10. The cleaned up solution is now ready for analysis. Please refer to Method section.

Sample Preparation

• Dried Fruit

This method has been tested on a number of dried fruits including sultanas, raisins, figs and apricots.

1. Weigh 50 g of ground sample into a 1 litre capacity, solvent resistant blender jar.
2. Add 100 ml of 100 % methanol and blend at high speed for 1 minute.
3. Add 100 ml of 1 % sodium bicarbonate and blend at high speed for a further 2 minutes.
4. Filter the sample through Whatman No. 113 or No. 4 filter paper, or centrifuge at 4,000 rpm for 10 minutes.
5. Dilute 12 ml of filtrate with 12 ml of PBS.
6. Dependent on the screening level required pass the appropriate volume of diluted filtrate through the immunoaffinity column according to the table below.

Screening Level	Volume of Diluted Filtrate Applied to Column
5 ppb	20 ml
10 ppb	10 ml

7. Pass the required volume of diluted filtrate through the immunoaffinity column at a flow rate of 2 ml per minute (or the sample can be allowed to pass through the column by gravity if preferred). A slow, steady flow rate is essential for the capture of the toxin by the antibody.
8. Wash the column by passing 10 ml of PBS through at a flow rate of approximately 5 ml per minute. Pass air though the column to remove residual liquid.
9. Elute the toxin from the column at a rate of 1 drop per second using 1 ml of 100 % methanol and collect in an amber glass vial. Backflushing is recommended. Please refer to the Backflushing section for further information.
10. Following elution pass 2 ml of sample diluent buffer through the column and collect in the same vial to give a 3 ml total volume.
11. 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.

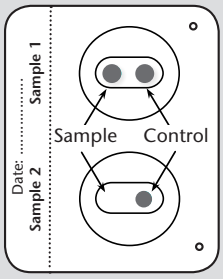
3. Once the sample has passed through the membrane apply 100 µl of ready-to-use conjugate (red label) and let it pass through the membrane.

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 8 hours of the first 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 and ISO 13485 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.

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