OCHRATOXIN-A IN GREEN COFFEE FLOW-THROUGH ASSAY

5127OTACG[1]08.23

A flow-through enzyme immunoassay for the detection of Ochratoxin-A in green coffee

EUROPROXIMA OCHRATOXIN-A IN GREEN COFFEE
FLOW-THROUGH RAPID TEST

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TABLE OF CONTENTS

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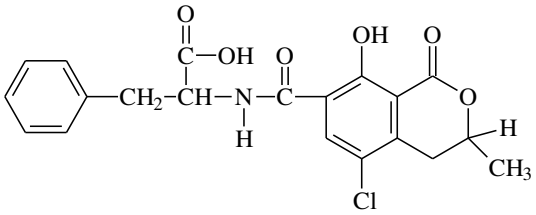
BRIEF INFORMATION

The Ochratoxin-A (OTA) Flow-Through Rapid Test is a competitive membrane-based enzyme immunoassay for the detection of OTA in green coffee. The test is based on mouse monoclonal antibodies against OTA. With this kit 10 analyses can be performed. The kit contains all the reagents required to perform the tests. The cut-off value of this test is 3 µg/kg of OTA in green coffee.

1. INTRODUCTION



OCHRATOXIN A

Ochratoxin A (OTA) is a nephrotoxic and hepatocarcinogenic mycotoxin produced by *Penicillium verrucosum* and *Penicillium viridicatum* in temperate and cold climates and by a number of *Aspergillus* species such as *A. ochraceus* in warmer and tropical areas of the world. OTA has been shown to occur in various cereals and plant products such as coffee and grapes. In the European Union the maximum limit for OTA in roasted coffee is 3 µg/kg in accordance with Commission Regulation (EU) 2022/1370. There is no maximum limit set for green coffee. In line with regulation for roasted coffee the cut-off value for green coffee was set to 3 µg/kg.

2. PRINCIPLE OF THE OCHRATOXIN-A FLOW-THROUGH ASSAY

The membrane-based assay kit consists of 10 devices each pre-coated with OTA-BSA (test line) anti-mouse IgG (control line). Specific antibodies (mouse anti-OTA) labelled with horseradish peroxidase (HRP-antibody conjugate) are supplied lyophilised in 10 separate vials. Green coffee extract is added to these reaction vials and pre-incubated for 5 minutes. During this incubation specific antibodies bind any OTA present in the sample. Then the mixture is transferred onto the membrane where any unbound antibodies bind to OTA-BSA on the test line and bound/unbound antibodies bind to the anti-mouse IgG on the control line. After the sample extract has been completely absorbed through the membrane the unbound HRP-antibody conjugate is removed by a washing step. A chromogen substrate (tetramethylbenzidine, TMB) is then added. Bound HRP transforms the chromogen substrate into a blue coloured product and this appears as a line. Two minutes after the addition of the substrate the background membrane colouration is washed away with a final washing step. The results are then visually interpreted.

3. HANDLING AND STORAGE

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

- After the expiry date has passed, the kit cannot be used.

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

- Avoid direct light on the substrate solution.

- If a blue colouring of the substrate solution is observed, it may indicate a degeneration and the component cannot be used for the test.

4. KIT CONTENTS

- 2 x 5 membrane devices
- 10 filters
- 10 syringes
- 10 vials containing methanol (2 ml) (Extract)
- 30 Pasteur pipettes (1 ml)
- 10 vials green coffee purification buffer (Purify)
- 10 reaction vials (React, black cap)
- 1 vial wash buffer (Wash, white cap)
- 1 vial substrate solution (Colour, blue cap)

5. EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- Pipette 100 – 1000 µl (recommended) (alternatively use 1 ml Pasteur pipettes provided in the kit)
- Vials for sample filtrate collection (glass or polypropylene tube, vial, cup, container; volume at least 3 ml)
- Timer/clock
- Camera/smartphone for taking photos of the results (if required)
- Gloves

6. PRECAUTIONS

- This kit may contain hazardous substances. For hazard notes please refer to the appropriate safety data sheets (SDS).
- 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.
- Do not use components past expiration date and do not use components from different lots.
- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain good precision and accuracy.

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°C to 8°C.
All the reagents are provided ready to use.

8. SAMPLE PREPARATION AND ASSAY PROCEDURE

Sample preparation

1. Add 1 gram of ground green coffee to a vial containing 2 ml of methanol (Extract). Shake by hand for 1 minute and leave the sample for 5 minutes. Repeat this twice more (total extraction time 15 min).

2. Use a Pasteur pipette provided in the kit to transfer at least 0.75 ml of the upper methanol layer to a clean tube. Let the extract stand until coffee slurry is settled to the bottom of the tube.

3. Add 0.5 ml of the clear methanol layer to a purification buffer vial (Purify). Use a Pasteur pipette provided in the kit to measure 0.5 ml or use a single channel pipette (100 µl – 1000 µl). Shake by hand for 1 minute.

4. Remove a plunger from a syringe and attach a filter to the syringe.

5. Mix the content of the buffer vial well and pour quickly into the syringe. Place the plunger back onto the syringe.

6. Filter the sample and collect the filtrate into a clean sample tube/container. The sample extract is ready to use in the test (stable for at least 1 hour).

Assay procedure

7. Add 0.75 ml of the filtered sample extract to a reaction vial (React). Use a Pasteur pipette provided in the kit to measure this 0.75 ml or use a single channel pipette (100 µl – 1000 µl). Mix by swirling and incubate for 5 minutes at room temperature.

8. Pour the content of the reaction vial onto the membrane window of a FTR device. Allow liquid to flow-through completely.

9. Add 5 drops of wash buffer (Wash). Allow liquid to flow-through completely.

10. Add 3 drops of substrate (Colour) and incubate for 2 minutes.

11. Add 2 drops of wash buffer (Wash, white cap) onto the device. Allow the liquid to flow-through completely

12. Read the result.

9. INTERPRETATION OF RESULTS

Short explanation

If the sample is negative for OTA (<3 µg/kg) then two blue coloured lines appear (C + T).
If the sample is positive for OTA (≥3 µg/kg) then only one blue coloured line (control line) appears (C).
The test is invalid when no blue coloured line appears. The sample should be retested.

Further considerations

The test gives positive result (only control line visible) if a sample contains at least 3 µg/kg of OTA.

Test line	Control line	Result interpretation
yes	yes	sample contains no OTA
yes/no (weak)	yes	sample can contain low level of OTA (below 3 µg/kg)
no	yes	sample contains ≥3 ug/kg of OTA

If the sample is found to be non-compliant, the results shall be verified by re-analysis of the sample using a confirmatory method.

10. LITERATURE

Commission Regulation (EU) 2022/1370 of 5 August 2022 amending Regulation (EC) No 1881/2006 as regards maximum levels of ochratoxin A in certain foodstuffs.

11. ORDERING INFORMATION

For ordering the Ochratoxin A wine FTD kit, please use cat. code: 5127OTACG.

12. REVISION HISTORY

Not applicable

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