QuickTox™ Kit for QuickScan
Ochratoxin-A in Green Coffee

Cat. # OTA-COFY1

User Manual
1 – Intended Use

This QuickTox Test protocol is validated on Green Coffee (Arabic Coffee and blends of Arabic and Robusta Coffee). The test provides quantitative results using QuickTox Ochratoxin-A kit on these matrices for residues ranging from 3 ppb to 30 ppb. The analysis must be performed using QuickScan Software in raw-signals mode and the dedicated calculation sheet “GENERON-Ochratoxin-A”.

2 – Contents of kit

• 50 QuickTox Strips packed in a moisture-resistant canister
• 50 reaction vials
• 100 pipette tips
• Modified Grain Buffer for Green Coffee Concentrate
• DB3 Buffer
• Available in 50-stripped individual kit format or bulk packaging

3 – How the Test Works

A composite sample is collected, ground and then extracted to solubilize any Ochratoxin-A present. Each sample should be ground and extracted with Modified Grain Buffer for Green Coffee preheated at 70°C. This extract is further diluted with DB3 Buffer for testing with the QuickTox Kit.

Each QuickTox Strip has an absorbent pad at each end. The protective tape with the arrow indicates which end of the strip to insert into the reaction vial. The sample extract travels up the membrane strip and is absorbed into the larger pad at the top of the strip. At ten minutes, the strip is cut off at the top of the arrow tape, the bottom pads are discarded, and the strip is inserted into the QuickScan reader to obtain raw signals. To have quantitative results the signals need to be inserted into “GENERON-Ochratoxin-A” spreadsheet.

4 – Preparation of the Sample

Please note:
• Sample extract should be tested immediately after dilution with DB3 Buffer. Make sure strips and DB3 Buffer are at room temperature and ready for use before the dilution step.
• The Grain Buffer Concentrate inserted in this product is modified. Don’t use the original Grain Buffer Concentrate from other kits.

Determine size of sample

1. Collect a composite sample according to your own sampling plan.
2. Grind samples using a grinder or mill which provides a sample with a consistency comparable to the standard setting of “Turkish” on a Bunn grinder. Mix ground material thoroughly before sub-sampling. The ground sample should pass through a 20-mesh sieve.
Prepare Modified Grain Buffer for Green Coffee

3. In a clean vessel with a cover, dilute the Modified Grain Buffer for Green Coffee Concentrate 20 fold using 19 parts of water distilled and/or deionized and one part concentrate. (For example: 25 mL Buffer for Green Coffee Concentrate into 475 mL water.) Mix to make homogenous and preheat (70°C) the volume required for analysis. Modified Grain Buffer for Green Coffee can be stored for up to 7 days at room temperature or may be stored refrigerated for longer life (note: bring Buffer to room temperature before testing).

Extract sample with Modified Grain Buffer for Green Coffee

4. Weigh 5 grams of milled sample into a 50mL conical centrifuge tubes sample cup with lid.
5. Add five volumes of preheated diluted 1X Modified Grain Buffer for Green Coffee at 70°C (i.e. 5 grams, add 25 mL).
6. Cap sample cup tightly and shake vigorously by hand for 2 minutes. Samples that are not thoroughly mixed may adversely affect test results due to incomplete extraction.
7. Incubate the sample according to one of the following procedures:
   • Incubate 10 minutes in thermostatic bath at 70 °C, shake vigorously by hand for 1 minutes at mid incubation (after 5 minutes from the start of incubation) and for 2 minutes at the end of incubation.
   • Alternatively, incubate 10 minutes in our dedicate thermomixer (code ACC2400) at 70°C and shaking at 800 rpm.
8. Centrifuge the tube or an aliquot of sample extract for 3 minutes at 2000 x g (not RPM). Consult centrifuge manual for g force calculation, and follow manufacturer’s instructions for operation and balancing.

Dilute sample with DB3 Buffer (use 2 separate pipette tips)

9. With a new pipette tip, transfer 50 μL of DB3 Buffer into the reaction vial. Discard tip.
10. Using another new pipette tip, remove 200 μL from middle layer of centrifuged sample and add to the reaction vial containing the DB3 Buffer and mix well with pipette by stirring or drawing liquids up and down in the pipette tip. Discard pipette tip.

NOTE: Samples that are not thoroughly mixed and/or accurately pipetted will adversely affect test results. After adding the sample, the final volume in the reaction vial should be 250 μL. Do not reuse diluted samples. Always use a new reaction vial and two pipette tips for each sample, and discard vials and tips after use.
5 – How to Run the QuickTox Strip Test

1. Allow refrigerated canisters to come to room temperature before opening. Remove the QuickTox Strips to be used. Avoid bending the strips. Reseal the canister immediately.
2. Place the strip into the reaction vial containing the DB3 Buffer and sample extract. The arrow tape on the end of the strip should point into the reaction vial.
3. Sample extract will travel up the strip (flow may not be visible immediately—this is expected and normal). Reaction vials will stand on their own.
4. Allow the strip to develop for 10 minutes. Immediately cut off and discard the bottom section of the strip covered by the arrow tape. Insert strip into the QuickScan reader for reading of raw data.

6 – Use of the QuickScan System

Remember: to perform this test you must have the QuickScan software in raw signals mode. If you have any doubt, please contact Generon S.p.A. (immunolab@generon.it).

Detailed instructions for use of the QuickScan system are supplied with each unit, and can also be found at www.envirologix.com/quickscan. In summary, a strip is inserted face down in the carrier with the barcoded end closest to the handle. The carrier is inserted into the reader and the strips are read by touching or clicking on the "Read Test" area of the screen. Is now possible view SI value in the corresponding cell.
7 – Calculation of results

For the calculation of results use "GENERON-Ochratoxin-A" spreadsheet.

1. Insert manually REF values provided in the batch Quality Control Certificate in the orange cells. These values are lot specific and cannot be applied to different lots.
2. Insert manually SI values obtained with the sample in the yellow cells.
3. The results in ppb are automatically calculated in the corresponding column.

Results are reported in the range of 3 to 30 ppb. Results less than 3 ppb are reported as "<3" and results greater than 30 ppb are reported as "> 30 ppb."

8 – Kit Storage

This QuickTox Kit should be stored refrigerated. Note the shelf life on the kit box. Prolonged exposure to high temperatures may adversely affect the test results. Do not open the desiccated canister until ready to use the strips.

9 – Disclaimers

Generon S.p.A. guarantees the buyer exclusively concerning the quality of reagents and of the components used to manufacture the product. Generon S.p.A. is not responsible, and cannot anyway be considered responsible or jointly responsible, for any possible damages resulting from the utilization of the product by the user and from the data obtained.