VERSION N2
CAT.NUMBER: S6324/S6348

SYMMETRIC OCHRATOXIN COFFEE

LATERAL FLOW TEST KIT

for the quantitative determination of Ochratoxin in Coffee

ProGnosis Biotech S.A. is ISO 9001:2015 certified by TÜV Hellas (TÜV NORD).

Use only the current version of Product Data Sheet enclosed with the kit.

Symmetric OCHRATOXIN Coffee, S6324/S6348, is a Lateral Flow Test kit for the quantitative determination of Ochratoxin A in coffee.

This kit contains all reagents required for 24 or 48 reactions.

Matrices:

Type I: Green coffee
Type II: Roasted coffee (Espresso, Filter, Greek)

DO NOT USE this kit for the detection of Ochratoxin in Instant coffee

- Test time (reaction time after samples and reagents preparation): 10min
- Range: 0 - 20ppb
- Shelf life: 12 months
- Storage: 2-8^{o}C

This is an electronic version, please verify always the last one included in the kit.

Specifications

- The LOD of the method is 0.6ppb OTA.
- The LOQ of the method is 0.9ppb OTA.
- Cross-reactivity: The cross-reaction of the anti-Ochratoxin antibody with Ochratoxin A and B is 100 and <0.1% respectively.

1. Description

Symmetric OCHRATOXIN Coffee is an innovative Lateral Flow test, utilizing state-of-the-art features for the quantitative detection of Ochratoxin A (OTA) in coffee. This Lateral Flow test utilizes an ecological solution for the extraction step.

2. General Information

Ochratoxins are a group of mycotoxins produced by some Aspergillus species (mainly *A. ochraceus*, but also by 33% of A. niger industrial strains) and some Penicillium species, especially *P. verrucosum* and *P. carbonarius*. Ochratoxin A (OTA) is the most prevalent and relevant fungal toxin of this group, while ochratoxins B and C are of lesser importance. OTA is a potent nephrotoxin and causes both acute and chronic effects in the kidneys of all mammalian species tested. It is also genotoxic (damages DNA) and teratogenic (damages the fetus) and is considered a probable carcinogen, causing renal carcinoma and other cancers in a number of animal species. Most controlling government agencies worldwide have set regulatory limits for the occurrence of Ochratoxin A (OTA) in foods cereals, products derived from cereals, dried vine fruits, roasted coffee, soluble coffee, wine and grape juice. Accurate and rapid determination of the presence of OTA in commodities is of paramount importance.

3. Principle of the Method

The quantitative lateral flow test is based on the immunochromatography assay principles. The wells of the microtiter strips contain OTA specific antibodies conjugated to colloidal gold. Diluted sample is added into the well. A dipstick with two capture lines, test and control, is dipped into the well. The suspended mixture starts flowing vertically on the dipstick and passes through the two lines. While running, OTA (if it is present) binds to the antibodies. A valid test should always have the upper control line red. If the sample is free of OTA, a color development occurs at the test line, indicating the absence of OTA in the sample. On the contrary, the presence of OTA in the sample will cause a reduced colored signal at the test line. The test line color intensity is indirectly proportionate to the concentration of OTA present in the samples. By utilizing S-Flow software and the symmetric quantification technology, OTA is accurately quantified.

4. Reagents Provided

Symmetric OCHRATOXIN Coffee kit contains sufficient reagents and materials for 24/48 reactions.

Reagents (Store at 2-8ºC)	Quantity for 24 reactions	Quantity for 48 reactions
Pots each with 1 strip of 8 reagent microwells and 8 dipsticks	3	6
Sample Diluent Tubes	24	48
High Range Solution (10ml)	1	1

5. Materials required but not provided

- A grinder sufficient to render sample to particle size of fine instant coffee
- Balance with 0 - 50g measuring capability and Graduated cylinder - 50ml
- 65% Ethanol
- Mini centrifuge (spin) and plastic tubes 1,5 or 2ml
- Tube roller or Vortex mixer
- 100 or 200μl adjustable micropipettes (single or multi channel) with disposable tips
- **S-Flow** software along with matching scanner device

6. Storage Instructions

Store kit components between 2 - 8°C. Do not freeze any components provided. Reseal the unused strips in the storing tube together with the desiccant bag provided. The expiry date of the kit and reagents is stated on their labels and no quality guarantee is accepted after the expiration date. The expiry of the kit components can only be guaranteed if the components are stored properly and the reagent is not contaminated due to prior handling. Do not interchange individual components between kits of different lot numbers.

7. Safety and Precautions for use

All reagents should be brought to room temperature (21 - 25°C) before use (at least half an hour) and covered when not in use. Use a clean disposable plastic pipette tip for each reagent, to avoid cross contamination.

8. Sample preparation

1. Prepare the Extraction Solution (65% Ethanol) by adding 35ml od distilled or deionized water to 65ml of ethanol.
2. The sample must be collected according to established sampling techniques. Grind a representative sample to the particle size of fine instant coffee (50% passes through a 20 mesh screen)
3. Weigh out a 5g ground portion of the sample and add 15ml of the Extraction Solution. Mix using a tube roller for 5 minutes (or vortex for 2min). **The ratio of sample to Extraction Solution is 1:3 (w/v).**
4. Allow the particulate matter to settle. Centrifuge 1ml of the extract for 2min using a mini centrifuge (spin). (The extracted sample should have pH value of 6.2 - 7.0. If the pH is less than 6.2, the pH should be neutralized using NaOH.)
5. Add 100μl of supernatant into the Sample Diluent Tube provided and mix well. Use the diluted filtrate within 30 minutes.

9. Method Procedure

1. Before opening the reagents, take the kit out of the fridge and wait until the temperature of the reagents reaches the ambient temperature.
2. Download and/or set the kit's **lot number**, as provided in the Quality Assurance Certificate and then chose the suitable **matrix type**.
3. Open one plastic pot and take out as many test strips and microwells as samples to be tested.
4. The pot with dipsticks should **always be well closed** after reagents have been taken out.
5. Dispense **100μl of diluted sample** into the microwell and pipette **up and down 4 times** to completely mix the lyophilized gold particles in the sample, while avoiding bubbles. The sample should turn into a **uniform pink color**. In case of more than 2 samples, an 8 channel multipipette should be used.
6. Place the appropriate number of sticks into microwells **immediately** and set timer for 10 minutes.
7. When the 10 minutes are over, take the dipsticks out of the microwells.
8. Remove the white cotton sample-pad of the stick **immediately**. Touch the stick with your hand from the colorful pad and remove the white pad with your hands. Do not use a paper, towel or any other material.
9. Place the stick inside the plastic holder in order to be scanned. In case of S-Flow & 3PR-MINI scanner, the sticks must be facing up. In case of EPSON scanner, the **sticks must be facing down (inverted)** and the colored side must be facing the orange sticker.
10. The software will use a Lot specific curve to calculate the results (ppb) . A simple visual interpretation of the stick is NOT possible.

10. Performance Evaluation

10.1 Reference Materials

Several reference materials are being used for the evaluation of each product of ProGnosis Biotech S.A. in the context of Quality Control performed by Quality Control Department. Please request a validation report, including the results, at info@prognosis-biotech.com.

10.2 Proficiency Tests

All products participate frequently in Proficiency Tests. For more information, visit the individual product page in our website: **www.prognosis-biotech.com**

11. Method Summary

Total method time: 10 minutes

Add 100µl of the extract into the Sample diluent tube provided

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Dispense 100µl of each sample into the microwells and mix 5 times the sample with the lyophilized gold particles

⇩

Place the appropriate number of sticks into microwells immediately.

⇩

(Wait 10 mins)
Take the stick out and remove the white sample-pad immediately

⇩

Place the stick in the appropriate device to be scanned

⇩

Quantify through s-flow software

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