VERSION N4
CAT.NUMBER: S6024/S6048

SYMMETRIC OCHRATOXIN GREEN

LATERAL FLOW TEST KIT

for the quantitative determination of Ochratoxin in grains, cereals and nuts.

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 Green, S6024/S6048, is a Lateral Flow Test kit for the quantitative determination of Ochratoxin A in grains, cereals and nuts.

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

Matrices:

Type I: Corn, Corn flour, Oats, Hazelnut, Sunflower seeds, Walnut, Cashews, Millet, Dried dates.
Type II: Wheat, Barley, Malt.

Type III: Almond, Buckwheat, Buckwheat flour, Dried fig, Dried prunes, Dried vine fruits, Pea flour, Peanut, Pistachio, Raisins, Rice, Sesame. (As well as type I & II matrices that are greater than 20ppb)

- Sample preparation: extraction
- Test time (reaction time after samples and reagents preparation): 3min
- Range: **0 - 20ppb** Type I & Type II**, 0-50ppb** Type III
- 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: 0,7ppb (Type I & Type II), 1,7ppb (Type III)
- The LOQ of the method is: 1ppb (Type I & Type II), 2,5ppb (Type III)
- 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 Green is an innovative Lateral Flow test, utilizing state -of-the-art features for the quantitative detection of Ochratoxin A in grains, cereals and nuts. This Lateral Flow test utilizes an ecological solution for the extraction step, instead of the usual organic solvents.

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 foetus) and is considered a probable carcinogen, causing renal carcinoma and other cancers in a number of animal species, Most controlling government agencies worldwide have regulations regarding the amount of aflatoxins allowable in human and animal foodstuffs. 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 extract 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 Green 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
Extraction Solution 10X (50ml)	1	2
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
- Deionized water
- 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

Let the reagents warm to room temperature (21 - 25°C) before the analysis (at least half an hour) and cover them when not in use. Use a clean disposable plastic pipette tip for each reagent, to avoid cross contamination.

8. Preparation of Extraction Solution

In case of the occurrence of crystals in the **Extraction Solution 10X**, the warming by gentle dismantling (using hands) of the crystals is needed. Pour entire content of the solution concentrate (50ml) into a clean 500ml graduated cylinder, rinse the vial with distilled or deionized water and pour the content again into the cylinder and fill to a final volume of 500ml with distilled or deionized water (50ml Extraction Solution 10X and 450ml deionized water). Mix gently to avoid foaming, transferring the final solution from cylinder to a clean bottle and back two times. The clean bottle with **1X Extraction Solution** working solution can be left out of the refrigerator during the method procedure and subsequent be stored 2 - 8°C for one year.

9. Sample preparation

1. 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).
2. Weigh out a 5g ground portion of the sample and add 15ml of the Extraction Solution (see 8). Mix using a tube roller for 5 minutes (or vortex for 2min). **The ratio of sample to Extraction Solution is 1:3 (w/v).**
3. 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.)

For Type I - Type II: run 100**µl** of extract (supernatant).

For Type III: add **100µl** of extract (supernatant) into the Sample diluent tube provided and mix well. **Use** the diluted extract within **30 minutes**.

Note: For **Type I - Type II** matrices greater than 20ppb and **Type III** matrices greater than 50ppb, first add **100µl** of extract (or supernatant) into the Sample diluent tube provided and mix well. To achieve a dilution factor of 5 or 10, add 100µl of the diluted sample into 400µl or 900µl of **High Range Solution** (respectively). **Use** the second dilution within **30 minutes**.

Choose **Type III** and set the suitable dilution factor to multiply the results by **5** or **10**.

DILUTION FACTOR 5 (Quantify from 12,5 up to 250ppb)
100µl of the diluted sample + 400µl of **High Range Solution**

DILUTION FACTOR 10 (Quantify from 2,5 up to 500ppb)
100µl of the diluted sample + 900µl of **High Range Solution**

10. 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 set the suitable **type** and **Dilution Factor**.
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 prepared 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 3 minutes.
7. When the 3 minutes are over, take the dipsticks out of the microwells and 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.
8. Place the stick inside the plastic holder in order to be scanned. In case of EPSON scanner, the **sticks must be facing down (inverted)** and the colored side must be facing the orange sticker. **NOTE:** The sticks should be scanned within 2 minutes after the sample-pads removal.
9. The software will use a Lot specific curve to calculate the results (ppb). A simple visual interpretation of the stick is NOT possible.

11. Performance Evaluation

11.1 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.

11.2 Proficiency Tests

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

12. Method Summary

Total method time: 3 minutes

Extract the samples

⇩

TYPE I & TYPE II

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

⇩

TYPE III

Add 100µl of extract (or supernatant) into the Sample diluent tube provided

⇩

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 3 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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