| VERSION 11

CAT.NUMBER: S5024/S5048

STORAGE: 2-8°C

SYMMETRIC ZON GREEN

LATERAL FLOW TEST KIT

for the quantitative detection of Zearalenone in grains, nuts, cereals and animal feed

PROGNOSIS BIOTECH

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

www.prognosis-biotech.com

This Lateral Flow test kit is manufactured by ProGnosis Biotech S.A.

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 ZON Green, S5024/S5048, is a Lateral Flow Test kit for the quantitative determination of Zearalenone in grains, cereals, nuts and animal feed.

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

Matrices:
Type I: Barley, Brown rice, Corn, DDGS, Oats, Rice, Wheat.

- Sample preparation: extraction
- Test time (incubation time after samples and reagents preparation): 5min
- Range: 0 - 800ppb
- Shelf life: 12 months
- Storage: 2-8^{o}C

1. Description

Symmetric ZON Green is an innovative Lateral Flow test, utilizing state-of-the-art features for the quantitative detection of Zearalenone in grains, cereals, nuts and animal feed. This Lateral Flow test utilizes an ecological solution [1] for the extraction step, instead of the usual organic solvents.

2. General Information

Zearalenone (ZON) is a member of the trichothecene mycotoxins produced by fungi of the Fusarium genus (F. graminearum). Grains including barley, wheat, oats, corn, rice and maize are frequently infected by this fungus. It is frequently implicated in reproductive disorders of farm animals and occasionally in hyperoestrogenic syndromes in humans. There is evidence that ZON and its metabolites possess oestrogenic activity in pigs, cattle and sheep. Moreover, ZON has also been shown to be hepatotoxic, haematotoxic, immunotoxic and genotoxic. Most controlling government agencies worldwide have regulations regarding the amount of ZON allowable in human and animal foodstuffs. Accurate and rapid determination of ZON presence 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 ZON 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, ZON (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 ZON, a color development occurs at the test line, indicating the absence of ZON in the sample. On the contrary, the presence of ZON 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 ZON present in the samples. By utilizing S-Flow software and the symmetric quantification technology [2, 3], ZON is accurately quantified.

4. Reagents Provided

Symmetric ZON Green kit contains sufficient reagents and materials for 24/48 measurements.

Reagents (Store at 2-8ºC)	Quantity for 24 wells	Quantity for 48 wells
Pots each with 1 strip of 8 reagent microwells and 8 dipsticks	3	6
Sample Diluent Tubes (8ml each)	24	48
Extraction Solution 10X (50ml)	1	2
High Range Solution (50ml)	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
- Filter Paper Whatman #1 or equivalent, Filter Funnel and Miscellaneous laboratory plastic or glass tubes 5 - 15ml
- Tube roller or Vortex mixer and **One-touch** Incubator for strips
- 200 or 300µl adjustable single channel micropipettes with disposable tips
- **S-Flow** software along with matching scanner device provided by lateral logic ltd

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. 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. 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 month.

Reagents needed	24 measurements	48 measurements
Pots each with 1 strip of 8 reagent microwells and 8 dipsticks	3	6
Extraction Solution 10X (50ml)	1	2

9. Sample Preparation

- 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).
- 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).**
- Allow the particulate matter to settle. Centrifuge 1ml of the extract for 2min using a mini centrifuge (spin). Alternatively, filter the extract through a Whatman #1 filter paper (or equivalent) and collect the filtrate.
- Add **200µl** of filtrate (or supernatant) into the Sample Diluent Tube provided (8ml) and mix well (41 times dilution). Run the diluted filtrate within 30 minutes.

NOTE 1: 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.

NOTE 2: If a range 0 - 4000ppb is required, mix the diluted sample with High Range Solution 1:4 (five times). Then, use only the 5X Dilution Matrix Type.

10. Method Procedure

1. Plug in the **One-touch** Incubator and wait until the temperature has been stabilized at 40°C.
2. Before opening the reagents, take the kit out of the fridge and wait until the temperature of the reagents reaches the ambient temperature.
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. Place the microwell(s) in the incubator.
6. Dispense **200µl of diluted filtrate** 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**.
7. Place the appropriate number of sticks into microwells immediately.
8. Push the START(RUN) button and a 5-minute countdown starts.
9. When the 5 minutes are over, i.e. after the sound-signal, take the dipsticks out of the microwells and press START (STOP)* again to stop the ringing tone.
10. Remove the white cotton sample-pad of the stick. 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.
11. 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.
12. Use S-flow software to quantify results within 10 minutes after the end of analysis. The software will use a Lot specific curve to calculate the results (ppb) according to the matrix sample type. A simple visual interpretation of the stick is NOT possible.

11. General Specifications

- The LOD of the method is 40ppb
- The LOQ of the method is 60ppb
- **Cross-reactivity:** The cross-reaction of the anti-ZON antibody with Zearalenone, α-zearalenol, β-zearalenol, Zearalanone, α-zearalanol and β-zearalanol is 100, 85, 47, 96, 80 and 82% respectively.
- **Matrices: Type I:** Barley, Brown rice, Corn, DDGS, Oats, Rice, Wheat.

12. Performance Evaluation

12.1 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.

12.2 Proficiency Tests

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

13. References

[1] M. Gkanas, Ch. Chatzoglou, K. Badra, Ch. Tsaridou, A.N. Ntantasios, G. Papageorgiou and S.D. Athanasiou, Uso di solventi non organici nell'analisi delle micotossine. Seminario AIA Laboratori e 20o ARAL SATA. 30-31 January 2018, Milan, Italy.

[2] Papageorgiou G, Ntantasios AN, Voulgari D, Badra K, Gotsopoulos M and Athanasiou SD, An innovative symmetric lateral flow system for the quantification of Aflatoxin M1. 8th International Symposium on RAFA, 7-10 November 2017, Prague, Czech Republic.

[3] Ntantasios AN, Arampatzis A, Voulgari D, Badra K, Papageorgiou G, Athanasiou SD and Gotsopoulos M, Innovative lateral flow method for the quantification of Aflatoxin M1. IDF DAIRY SUMMIT, 29 October-03 November 2017, Belfast, Northern Ireland, UK.

All immune assays supplied by ProGnosis Biotech S.A., are warranted to meet or exceed our published specification when used under normal conditions in your laboratory. If the product fails during the stated period, a replacement product will be issued.

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