VERSION N4
CAT.NUMBER: S4024/S4048

SYMMETRIC DON GREEN

LATERAL FLOW TEST KIT

for the quantitative determination of Deoxynivalenol in grains, cereals and animal feed

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 DON Green, S4024/S4048, is a Lateral Flow Test kit for the quantitative determination of Deoxynivalenol in grains, cereals and animal feed.

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

Matrices:

Type I: Corn, Corn Germ, Corn Germ Meal*, Corn flour, Corn Silage, Wheat germ, Toasted wheat germ, Barley, Malt, Oats, Soybeans, Soybean Meal, , DDGS, DDGS Molasses, Sorghum, Sunflower meal, White Rice, Brown Rice, Rice flour, Buckwheat, Cotton-seed, Millet, Beer residue, Pasta, Dried Palm, Pea flour, Dried Gai Choy, Dried Brassica Integrifolia

Type II: Wheat, Wheat flour, Rye*, Rye Flour*

- Sample preparation: extraction
- Test time (reaction time after samples and reagents preparation): 3min
- Range: 0 - 5ppm
- Shelf life: 12 months
- Storage: 2-8^{o}C

**Contact our company for extraction procedure*

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

Specifications

- The LOD of the method is 0.1ppm DON.
- The LOQ of the method is 0.15ppm DON.
- Cross-reactivity: The cross-reaction of the anti-DON antibody with 15-acetyl-DON, DON and 3-acetyl-DON is >100, 100, <0.1% respectively.

1. Description

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

2. General Information

Deoxynivalenol (DON), also known as vomitoxin, is a member of the trichothecene mycotoxins produced by fungi of the Fusarium genus (*F. graminearum*). Grains including barley, wheat, oats, corn and maize are frequently infected by this fungus. Deoxynivalenol, along with 3-acetyl- and 15-acetyl-DON, constitutes a highly toxic molecule and it is considered to play a crucial role in immunological and nervous system problems. Due to their cytotoxicity, these toxins will always be a risk to human and animal health. Most controlling government agencies worldwide have regulations regarding the amount of DON allowable in human and animal foodstuffs. Accurate and rapid determination of DON 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 DON 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, DON (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 DON, a color development occurs at the test line, indicating the absence of DON in the sample. On the contrary, the presence of DON 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 DON present in the samples. By utilizing S-Flow software and the symmetric quantification technology, DON is accurately quantified.

4. Reagents Provided

Symmetric DON 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). To achieve good homogenization, ensuring that any portion of the sample will be representative of the whole, weight at least 20gr of the sample.**
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.)
4. Add **100µl** of extract (supernatant) into the Sample diluent tube provided and mix well. Use the diluted extract within **30 minutes**.

Note: In case the result is greater than 5000 ppb, the sample should be further diluted with **High Range Solution** and re-tested. 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** and set the suitable dilution factor to multiply the results by **5** or **10**.

DILUTION FACTOR 5 (Quantify from 0,75 up to 25ppm)
100µl of the diluted sample + 400µl of **High Range Solution**

DILUTION FACTOR 10 (Quantify from 1.5up to 50ppm)
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 **Dilution Factor type** and current **Room Temperature** (ºC).
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 extract** 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: 3minutes

Extract the samples

⇩

Add 100µl of extract (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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