VERSION N3
CAT.NUMBER: S8024/S8048

SYMMETRIC T-2/HT-2 GREEN

LATERAL FLOW TEST KIT

for the quantitative determination of T-2/HT-2 in grains and cereals

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 T-2/HT-2 Green, S8024/S8048, is a Lateral Flow Test kit for the quantitative determination of T-2/HT-2 in grains and cereals.

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

Matrices:

Type I: Corn, Barley, Oats, White Rice, Brown Rice.
Type II: Wheat.

Type III: Corn flour, Wheat flour, Soya, Millet. (as well as types I & II matrices that are greater than 600ppb)

- Sample preparation: extraction
- Test time (reaction time after samples and reagents preparation): 3min
- Range: **0 - 600ppb** Type I & Type II, **0-2000ppb** 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: 26.5ppb (Type I & Type II), 67ppb (Type III)
- The LOQ of the method is: 40ppb (Type I & Type II), 100ppb (Type III)
- Cross-reactivity: The cross-reaction of the anti-T-2 antibody with HT-2, T-2 Triol and T-2 Tetraol is 80, 3.7 and <0.1% respectively.

1. Description

Symmetric T-2/HT-2 Green is an innovative Lateral Flow test, utilizing state-of-the-art features for the quantitative detection of T-2/HT-2 in grains and cereals. This Lateral Flow test utilizes an ecological solution for the extraction step, instead of the usual organic solvents.

2. General Information

T-2 and HT-2 toxin belong to the group of trichothecenes. This group of mycotoxins are produced mainly by fungi of the genus *Fusarium* which is toxic to humans and animals. Agricultural commodities are frequently infected by this fungus. It is frequently implicated in cytotoxic and immunosuppressive disorders of farm animals and occasionally in pathogenetic syndromes in humans. Both in man and in animals T-2/HT-2 toxins can cause alimentary toxic aleukia. Most controlling government agencies worldwide have regulations or recommendations regarding the amount of T-2/HT-2 allowable in human and animal foodstuffs. Accurate and rapid determination of T-2/HT-2 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 T-2/HT-2 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, T-2/HT-2 (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 T-2/HT-2, a color development occurs at the test line, indicating the absence of T-2/HT-2 in the sample. On the contrary, the presence of T-2/HT-2 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 T-2/HT-2 present in the samples. By utilizing S-Flow software and the symmetric quantification technology, T-2/HT-2 is accurately quantified.

4. Reagents Provided

Symmetric T-2/HT-2 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	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-Type IV: 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 600ppb and **Type III** matrices greater than 2000ppb, first add **100µl** of filtrate (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 type to multiply the results by **5** or **10**.

DILUTION FACTOR 5 (Quantify from 0.5 up to 1ppm)
100µl of the diluted sample + 400µl of **High Range Solution**

DILUTION FACTOR 10 (Quantify from 1 up to 20ppm)
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

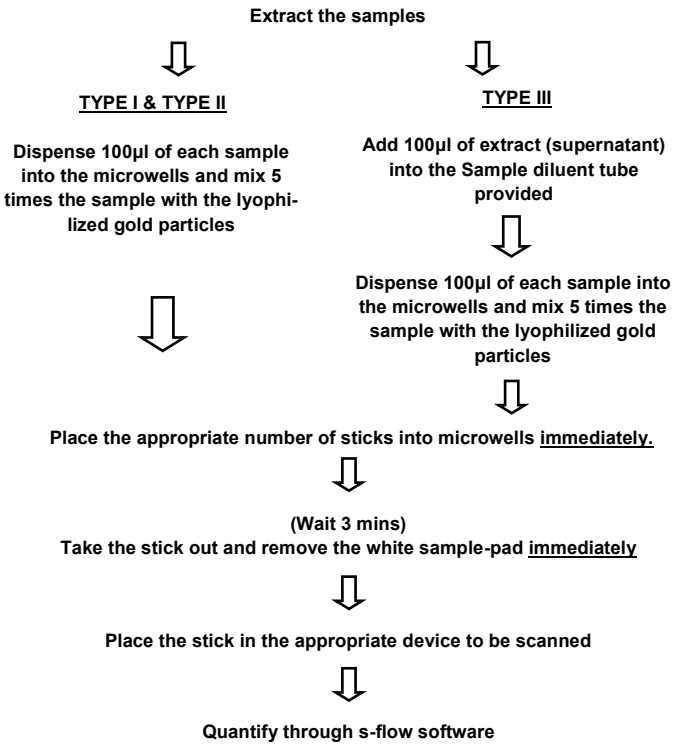
Several reference materials are being used for the evaluation of each product of ProGnosis Biotech S.A. in the context of Quality Control performed by Quality Control Department. Please request a validation report, including the results, at info@prognosis-biotech.com.

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



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