

Product Manual

AgraStrip® Pro T-2/HT-2 WATEX®

Part #: 10006614

Intended use

The AgraStrip® Pro T-2/HT-2 WATEX® test kit is an immunochromatographic assay designed for the quantitative analysis of the presence of T-2/HT-2 in food and feed components.

Performance characteristics

Range of quantitation: 0 – 1000 ppb

Limit of detection (LOD): 15 ppb (corn)

Limit of quantitation (LOQ): 20 ppb (corn)

Assay time: approx. 4 minutes

About T-2/HT-2

T-2 toxin (T-2) and its deacetylated form HT-2 toxin (HT-2, lacking the acetyl group at C-4) belong to the trichothecenes, a large family of mycotoxins that share a common tetracyclic ring system. *Fusarium langsethiae*, *F. poae*, and *F. sporotrichioides* are the predominant species that invade cereal crops and produce T-2 toxin and HT-2 toxin under cool and moist conditions on the field. Like most trichothecenes, T-2/HT-2 toxin can have acute as well as chronic toxic effects on humans and animals. As with other trichothecenes, T-2 and HT-2 toxins inhibit protein synthesis, a process followed by a secondary disruption of DNA and RNA synthesis (Ueno, 1984). These toxins further affect actively dividing cells such as those lining the gastrointestinal tract, as well as skin, lymphoid and erythroid cells. It can decrease antibody levels, immunoglobulins, and certain other humoral factors. The effects include weight loss or poor weight gain, bloody diarrhea, dermal necrosis or beak lesions, hemorrhage, and decreased production (weight gain, eggs, milk, etc.).

Product information

About AgraStrip® Pro T-2/HT-2 WATEX®

The AgraStrip® Pro T-2/HT-2 WATEX® test is a quantitative, competitive immunochromatographic assay designed to determine the presence of T-2/HT-2 toxin in a given sample. It is specifically tailored for testing grains, cereals, and other commodities.

Storage information

Upon receipt, promptly transfer the AgraStrip® Pro Total T-2/HT-2 WATEX® strips and dilution buffer to a refrigerated storage, maintaining a storage temperature of 2 – 8 °C (35 – 46 °F) when not in use. Extraction buffer bags and consumables can be stored at room temperature, 15 – 25 °C (59 – 77 °F). Avoid freezing the components. Refrain from using the kit after the expiration date marked on the label.

Contents of the kit

The AgraStrip® Pro T-2/HT-2 WATEX® test kit contains the following items:

- 1 can containing 40 AgraStrip® Pro T-2/HT-2 WATEX® strips
- 1 bag containing 40 AgraStrip® Pro cartridges (10006165)
- 1 foil bag containing 40 AgraStrip® Pro WATEX® extraction buffer bags
- 1 bottle of 50 ml AgraStrip® Pro T-2/HT-2 WATEX® dilution buffer
- 2 bags of 40 yellow or white pipette tips each
- 2 bags of 40 blue pipette tips each
- 4 bags of 20 microcentrifuge tubes (dilution tubes) each
- 4 rolls of 10 pieces of Whirl-Pak® filter bags each
- 1 tube holder
- 1 QR card for the AgraVision® Pro reader

Materials required but not included

Extraction procedure:

- Analytical balance with a weighing capacity up to 400 g
- Graduated cylinder (10002612) with a minimum capacity of 100 mL
- Distilled, de-ionized or bottled water for extraction
- Centrifuge

Assay procedure:

- 10006324: AgraVision™ Pro reader
- Single channel pipettes capable of pipetting up to 1000 µl or below fixed volume pipettes:
 - Fixed 100 µl pipette
 - Fixed 1000 µl pipette

AgraStrip® Pro T-2/HT-2 WATEX® test – assay principle

The AgraStrip® Pro T-2/HT-2 WATEX® test is a one-step lateral flow immuno-chromatographic assay designed for the quantitative screening of T-2 toxin and HT-2 toxin (T-2/HT-2) in samples. It operates on a competition immunoassay principle. During the test, the sample extract migrates through the conjugate pad containing T-2/HT-2 specific monoclonal antibodies conjugated to colloidal gold nanoparticles. If the sample contains T-2/HT-2, these mycotoxins will bind to the nanoparticle-antibody complex (conjugate) and migrate to the detection zone.

In this competitive format, the test line consists of immobilized analyte molecules (in this case, T-2/HT-2) conjugated to a protein carrier. Any unbound T-2/HT-2 antibody will be captured in the test zone forming a visible line. As the T-2/HT-2 concentration in the sample increases, the T-2/HT-2 mycotoxin will be captured by the antibody-gold particles and reduce the interaction of antibody-gold particle with the test zone analyte, resulting in a reduced signal at the test line. The color intensity of the line is therefore inversely proportional to the concentration of T-2/HT-2 in the sample. The control line indicates proper flow through the strip and should always be visible in the control zone, irrespective of the presence/absence of T-2/HT-2. The test strips are measured using the AgraVision™ Pro reader, which quantifies the concentration of T-2/HT-2 in the sample.

Protocol at a glance

This section offers a brief overview of the AgraStrip® Pro test procedure utilizing the AgraVision™ Pro reader. It is essential to read the complete product manual thoroughly before initiating the assay.



Turn on the AgraVision™ Pro reader by plugging it in and follow the setup procedure according to the instructions.



Pipette 200 µL dilution buffer into a fresh microcentrifuge tube.



Weigh in 10 ± 0.1 g of adequately ground representative sample and add one extraction buffer bag into a Whirl-Pak® filter bag, preferably on the same side as the sample.



Add 200 µL of extract supernatant to the dilution buffer. Mix well. Centrifuge the diluted sample extract for 30 ± 5 seconds.



Add 50 ml of distilled, deionized, or bottled water to the Whirl-Pak® filter bag.



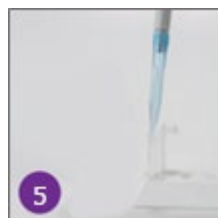
Insert an AgraStrip® Pro test strip into an AgraVision™ Pro cartridge. Transfer the cartridge containing the test strip into a port of the reader.



Seal the bag tightly and shake vigorously for 2 minutes $\pm$ 10 seconds. Then allow the sample to settle for 1 minute $\pm$ 10 seconds.



Enter sample ID and select matrix and quantitation range.



Precentrifugation step: Transfer 1000 µL of sample extract from the filter bag to a microcentrifuge tube. Take the extract from the side opposite to where the sample was weighed. Centrifuge the extract for 30 ± 5 seconds at 2000 g.



Add 200 µL sample to the AgraVision™ Pro cartridge.

AgraStrip® Pro WATEX® test procedure

Before starting

Procedural guidelines:

- Make sure you have everything you need ready before starting the test.
- All reagents and kit components must be allowed to reach room temperature before use.
- Do not return unused reagents into their original bottles.

Precautions:

- Store AgraStrip® Pro WATEX® test strips and dilution buffer at 2 – 8 °C (35 – 46 °F) when not in use, and do not use beyond the expiration date.
- Test strips must be kept inside their original tubes.
- Adhere to the instructions of the test procedures.
- Do not re-use test strips.
- Do not re-use AgraVision™ Pro cartridges.
- Treat all materials, containers, and devices that are exposed to the sample or standards as if they were contaminated with toxin.
- The components in this test kit have been quality control tested as a standard batch unit. Do not mix components from different lot numbers.
- Dispose all single-use materials, containers, and devices appropriately after use.

Sample preparation

The sample extraction is applicable to all AgraStrip® Pro WATEX® products (One for All WATEX®)

1. Obtain a representative sample of the specimen you want to analyze and grind it so that 95% will pass through a 20-mesh sieve (sieve opening 0.84 mm), then thoroughly mix the subsample portion.
2. Weigh out 10 ± 0.1 g of ground sample into one side of a Whirl-Pak® filter bag.
3. Take one extraction buffer bag from the foil pouch and place it on the same side of the Whirl-Pak® filter bag as the sample. The extraction buffer bag will dissolve completely during the extraction process.
4. Add 50 ml of distilled, deionized, or bottled water and close the WhirlPak® filter bag.
5. Vigorously shake for 2 minutes ± 10 seconds.
6. Open the Whirl-Pak® filter bag and place the filter on the same side as the sample.
7. Allow sample to settle for 1 minute ± 10 seconds.

AgraVision™ Pro reader setup

Turn the AgraVision™ Pro reader on prior to the test run. Choose the appropriate testing target (mycotoxins). The instrument will automatically heat up to the temperature needed to run the specific test.

Note: Do not insert the AgraStrip® Pro test strip until the instrument prompts you to do so.

Prior to the test run, insert the QR card, which contains information specific to the test kit's lot, into the reader (the slot is centrally located in the port system). After clicking the save icon, the QR code information will be stored. Each QR code only needs to be scanned once per lot.

Note: We recommend screening and saving all QR cards for new test kit lots prior to running the test.

Note: For detailed instructions see the AgraVision™ Pro user manual.

Extract preparation and dilution

1. Pipette 1000 µl extract into a microcentrifuge tube. The extract should be pipetted from the side of the filter bag opposite the ground sample. If the sample has a large foam head, tilt the bag for easier access to the supernatant below. The extract supernatant must be centrifuged for 30 ± 5 seconds at 2000 g using an appropriate device, such as a microcentrifuge.
2. Add 200 µL dilution buffer to a fresh microcentrifuge tube using a yellow or white pipette tip, and then add 200 µL supernatant from the centrifuged sample extract using a yellow or white pipette tip into the dilution buffer and mix well. This represents a 1:2 dilution.
3. The diluted sample must be centrifuged for 30 ± 5 seconds at 2000 g using an appropriate device, such as a microcentrifuge. Then, carefully take the supernatant for further testing.

Note: Diluted samples must be used within one hour, otherwise they are no longer effective.

Note: Make sure the pipette tip has been completely emptied.

Assay: quantitation range 1 (0 – 300 ppb)

1. Insert an AgraStrip® Pro test strip into an AgraVision™ Pro cartridge.
2. Insert the AgraVision™ Pro cartridge containing the test strip into any empty port/slot within the AgraVision™ Pro reader.
3. The reader automatically detects the type of test and the lot ID from the data matrix code on the test strip.
4. Enter a sample ID and choose the quantitation range and sample matrix then press enter (tick mark). Wait until a flashing drop prompts you to add the sample. Add 200 µl of the diluted centrifuged extract into the back of the AgraVision™ Pro cartridge.
The AgraVision™ Pro reader detects the fluid in the cartridge and will automatically start the timer for the incubation. Additional samples can be tested using the other 3 available slots of the system in parallel.
5. The test results will be automatically displayed on the relevant slot of the AgraVision™ Pro reader.

Assay: quantitation range 2 (200 – 1000 ppb)

If the reader detects a concentration greater than 300 ppb, the previously diluted sample extract (diluted extract from quantitation range 1) can be further diluted at a ratio of 1:4 (i.e., use a blue pipette tip to add 900 µL of dilution buffer to a microcentrifuge tube. Use a yellow or white pipette tip to add 300 µL of previously diluted extract to the dilution buffer). An additional measurement should be performed by repeating steps (1) – (5) of the assay (quantitation range 1). Please select the second quantitation range when entering the test information on the AgraVision™ Pro reader.

Limit of detection (LOD): 15 ppb (corn)

Limit of quantitation (LOQ): 20 ppb (corn)

Range of quantitation 1: 0 – 300 ppb

Range of quantitation 2: 200 – 1000 ppb

Note: AgraStrip® Pro T-2/HT-2 WATEX® tests give quantitative results in the above defined quantitation range. For example, if the quantitation range 1 (0 – 300 ppb) is selected and the test result is 350 ppb, it will be displayed as > 300 ppb. If quantitation range 2 (200 – 1000 ppb) is selected and the result is lower than 200 ppb, it will be reported as < 200 ppb.

Technical support

Uncertain if the test is compatible with your specific samples or matrices? Benefit from our extensive experience in mycotoxin testing. Reach out to the technical sales representative in your region for more information.

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