Version: v04
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Product Manual

AgraQuant® T-2/HT-2 (24/500) ELISA kit
Article number 10006953/10006954

Intended use
The AgraQuant® T-2/HT-2 (24/500) ELISA test kit is an immunoassay designed for the quantitative analysis of the presence of T-2 /HT-2 in food and feed components.
This product is intended for laboratory use.

Performance characteristics

Limit of detection (LOD): 24 ppb *

Limit of quantification (LOQ): 24 ppb *

Range of quantification: 24-500 ppb

Plate format: 96 (10006953) or 48 wells (10006954)

Assay time: 20 minutes

* Determined in corn.

About T-2/HT-2
T-2 toxin (T2) and its deacetylated form HT-2 toxin (HT2, lacking the acetylgroup 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 and HT-2 under cool and moist conditions on the field. Similar to most trichothecenes, T-2/HT-2 can have acute as well as chronic toxic effects on humans and animals. 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 the ELISA test kit

The AgraQuant® T-2/HT-2 assay is a direct competitive enzyme-linked immunosorbent assay (ELISA) that quantitatively determines T-2/HT-2 presence. This product is intended for use in grains, cereals, and other commodities.

Storage information

Upon receipt, immediately transfer the AgraQuant® T-2/HT-2 (24/500) test kit to refrigerated storage and keep it at 2-8°C (35-46°F) when not in use. Do not freeze. Do not use the kit beyond the expiration date indicated on the package.

Contents of the kit

The AgraQuant® T-2/HT-2 (24/500) ELISA test kit contains the following items:

10006953

- 96 antibody-coated microwells (12 eight-well strips) in a microwell holder sealed in a foil pouch
- 96 non-coated dilution microwells (12 white eight-well strips)
- 5 vials of 1.5 mL each of T-2/HT-2 standard (0, 24, 120; 240 and 500 ppb)
- 1 green-capped bottle of 25 mL of T-2/HT-2 toxin-conjugate solution
- 1 blue-capped bottle of 15 mL of substrate solution
- 1 red-capped bottle of 15 mL of stop solution
- 1 white-capped bottle of 25 ml 20x wash buffer (20x concentrated)

10006954

- 48 antibody-coated microwells (6 eight-well strips) in a microwell holder sealed in a foil pouch
- 48 non-coated dilution microwells (6 white eight-well strips)
- 5 vials of 0.75 mL each of T-2 /HT-2 standard (0, 24, 120, 240 and 500 ppb)
- 1 green-capped bottle of 12.5 mL of T-2/HT-2 toxin-conjugate solution
- 1 blue-capped bottle of 7.5 mL of substrate solution
- 1 red-capped bottle of 7.5 mL of stop solution
- 1 white-capped bottle of 25 ml 20x wash buffer (20x concentrated)

Materials required but not included

Extraction procedure:

- Grinding mill
- Blender or a tightly sealing jar with lid
- Analytical balance with a weighing capacity up to 200 g
- Graduated cylinder with a minimum capacity of 100 mL
- 50% (50:50 methanol:water) methanol or ACS grade methanol and distilled or deionized water for preparing a 50% methanol solution
- Container with a minimum capacity of 125 mL
- Whatman#1 filter paper, or equivalent
- Filter funnel

Assay procedure:

- Calibrated 8-channel and single-channel pipettes with 100 µL and 200 µL disposable plastic tips
- Timer
- Wash bottle
- Distilled or deionized water
- Absorbent paper towels
- 3 reagent boats for use as reagent containers for an 8-channel pipette
- Microwell reader with 450 nm and 630 nm filters

Visit **www.romerlabs.com** or get in touch with your technical sales representative to find out which of these items are also available from Romer Labs.

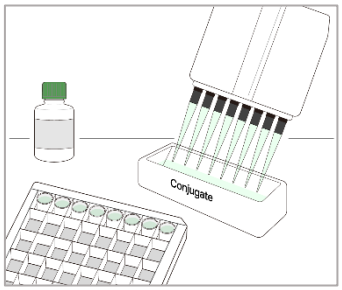
ELISA kit – Assay principle

The AgraQuant® T-2/HT-2 assay is a direct competitive enzyme-linked immunosorbent assay (ELISA). T-2/HT-2 is extracted from a ground sample with 50% methanol. The extracted, diluted sample (1:5 dilution factor) and enzyme-conjugated toxin are then mixed and added to the antibody-coated microwell. T-2/HT-2 in the samples or standards is allowed to compete with enzyme-conjugated toxin for the antibody binding sites. After a washing step, the enzyme substrate is added, which results in color development. The intensity of the color is inversely proportional to the concentration of T-2/HT-2 in the sample or standard. A stop solution is then added, which changes the color from blue to yellow. The absorbance of each well is measured at 450 nm and with a differential filter at 630 nm. The measurement must take place within 10 minutes after adding the stop solution.
To analyze the results, please refer to the Results analysis section at the end of this manual.

Protocol at a glance

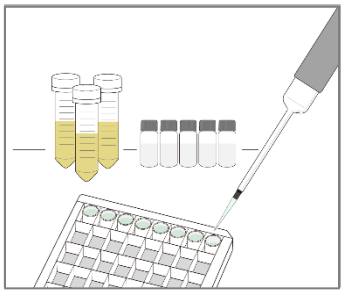
The following section provides an overview of the ELISA procedure. Before performing the assay, please read this product manual carefully.

1



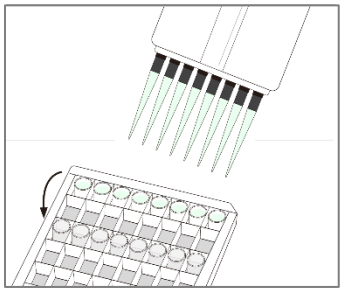
Pipette **200 µL of conjugate solution** into the dilution wells.

2



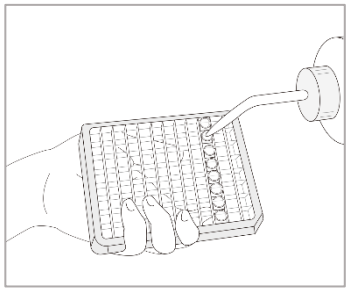
Add **100 µL of each standard or diluted sample extract** into the dilution wells.

3



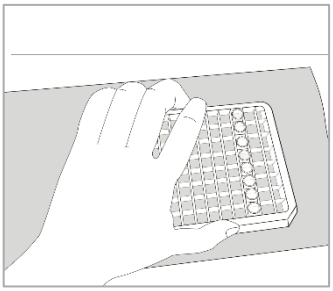
Mix well and **transfer 100 µL** from dilution wells into antibody-coated wells. **Incubate at RT for 15 minutes.**

4



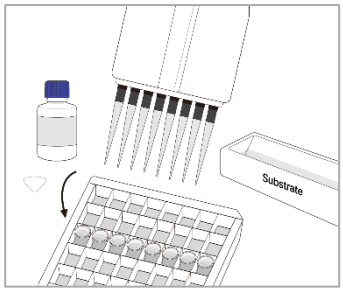
Wash 5 times with wash buffer.

5



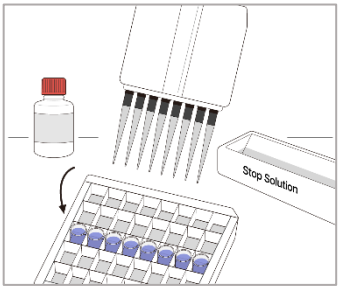
Tap dry the washed wells.

6



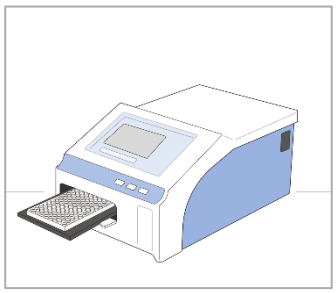
Pipette **100 µL of substrate** solution into the antibody-coated wells. **Incubate at RT for 5 minutes.**

7



Pipette **100 µL of stop solution** into the antibody-coated wells.

8



Read the absorbance of each well at **450 nm** with a differential filter at **630 nm.**

Reagent and sample preparation

Sample preparation

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 20 g of ground sample into a clean jar that can be tightly sealed.
3. Add 80 mL of 50% methanol extraction solution and seal the jar (1 part of the respective sample and 4 parts of extraction solution in a total of 5 parts)
4. Vigorously shake or blend for 3 minutes.
5. Allow sample to settle, then filter the top layer of extract through a Whatman #1 filter and collect the filtrate. Alternatively, centrifuge the extract 4 minutes at 3000 g using an appropriate device. **Note: Excessive pH values (<3 and >9) may require pH adjustment prior dilution.**
6. Dilute the sample extract 1:5 with deionized or distilled water. For example, add 1 mL of extract to 4 mL of distilled or deionized water.
7. Samples are ready for testing. Please read the ELISA procedure section and carefully follow the protocol.

Wash buffer preparation

Transfer the 25 mL of wash buffer 20x concentrated to a 500 mL plastic squeeze bottle. Add 475 mL distilled or deionized water and mix. We recommend using the ready to use (diluted) buffer within one month (stored at room temperature).

ELISA procedure

Before starting

Procedural guidelines:

- Make sure you have everything you need ready before starting the assay.
- All reagents and kit components must be allowed to reach room temperature, i.e., 18-30°C (64-86°F), before use.
- Run a standard curve with each assay.
- Adhere to the incubation times stated in the procedure. Use of incubation times other than those specified may give inaccurate results.
- We strongly recommended to perform the assay with an 8-channel pipette.
- Do not run more than 6 eight-well strips in one experiment when using an 8-channel pipette. If an 8-channel pipette is not used (i.e., using only single channel pipettes), we recommend that you run no more than a total of 16 samples and standards (2 test strips) in any one experiment.
- Do not return unused reagents into their original bottles.

Precautions:

- Store reagents at 2-8°C (35-46°F) when not in use, and do not use beyond the expiration date.
- Methanol is flammable. Caution must be taken in its use and storage.
- The stop solution contains acid. Avoid contact with skin or eyes. If exposed, flush with water.
- Treat all materials, containers and devices that are exposed to the sample or standards as if they were contaminated with toxin.
- Wear protective gloves and safety glasses when using the kit.
- Dispose of all single-use materials, containers, and devices appropriately after use.

Assay protocol

1. Place the appropriate number of **white dilution wells** in a microwell strip holder. One dilution well will be required for each standard (i.e., 0, 24, 120, 240 and 500 ppb) or sample.
2. Place an equal number of **antibody-coated microwells** in a microwell strip holder. Return unused microwells to the foil pouch with the desiccant packet and reseal pouch with tape.
3. Measure the required amount of conjugate solution from the green-capped bottle (~240 µL/well or 2 mL/strip) and place it in a separate container (e.g., reagent boat when using the 8-channel pipette). Using an 8-channel pipette, dispense **200 µL of conjugate solution** into each dilution well.
   ➲ **Did you know?** The ratio of conjugate to standard/sample should remain at a ratio of 2+1, but the volumes of conjugate and standards/samples can be reduced, e.g., using 100 µL and 50 µL, respectively. The content to be transferred from dilution wells to antibody-coated wells must remain at 100 µL.
4. Using a single channel pipette, add **100 µL of each standard or diluted sample extract** into the dilution wells containing 200 µL of conjugate. Use a fresh pipette tip for each standard or sample. **Note:** Make sure the pipette tip has been completely emptied.

5. Using an 8-channel pipette with fresh tips for each 8-well strip, mix each well by carefully pipetting up and down 3 times and immediately **transfer 100 µL of the content** of each dilution well into a corresponding antibody-coated microwell. Incubate at room temperature for **15 minutes.**

   Note: Do not attempt to mix the content of the microwells by shaking the plate as this may cause well-to-well contamination.

6. Empty the content of the microwell strips into a waste container. **Wash** by filling each microwell with wash buffer, and then dumping the wash buffer from the microwell strips. Repeat this step 4 times for a total of 5 washes.

   Note: Take care not to dislodge the strips from the holder during the washing steps.

7. Lay several layers of absorbent paper towels on a flat surface and tap the microwell strips on towels to remove as much residual wash buffer as possible after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.

   Note: Never insert absorbent paper directly into the wells.

8. Measure the required amount of substrate solution from the blue-capped bottle (~120 µL/well or 1 mL/strip) and dispense it into a separate container (e.g., reagent boat for an 8-channel pipette). **Pipette 100 µL of the substrate solution** into each microwell using an 8-channel pipette. Incubate at room temperature in the dark for **5 minutes.**

9. Measure the required amount of stop solution from the red-capped bottle (~120 µL/well or 1 mL/strip) and dispense into a separate container (e.g., reagent boat for an 8-channel pipette). **Pipette 100 µL of stop solution** into each microwell using an 8-channel pipette. The color should change from blue to yellow.

10. Read the absorbance of each well within 10 minutes after the addition of the stop solution at **450 nm** (reference wavelength 630 nm) with a microwell reader.

    Note: Carefully remove any air bubbles prior to reading the absorbance as they may affect the result.

    Note: Do not return unused reagents to their original bottles. Carefully note which rows/strips contain standards or samples during the assay.

Results analysis

Results can be easily calculated using the **Romer Labs spreadsheet** that is provided free of charge upon request. With the Romer Labs spreadsheet, you only need to insert the obtained OD values for standards and samples. The spreadsheet applies the Log/Logit regression model to construct a calibration curve. The correlation coefficient (R) of the calibration curve must be between -0.990 and -1.000. The T-2/HT-2 concentration in your samples is calculated automatically by interpolation with the calibration curve.
Alternatively, construct a dose-response curve using either the unmodified OD values of the standards or the OD values expressed as a percentage of the OD of the zero (0) standard.
When working according to the sample preparation section described in this package insert, the extraction factor (ratio of 1:4) and dilution factor of 1:5 is applied during sample extraction. The extraction and dilution factors are already taken into account; thus the T-2/HT-2 concentration can be read directly from the standard curve obtained.

Note: If a sample contains T-2/HT-2 levels higher than the highest standard (>500 ppb), the filtered extract should be further diluted with distilled or deionized water such that the diluted sample results in a range of 24-500 ppb. The diluted sample should be reanalyzed to obtain accurate results. The applied dilution factor must be included in the calculation of the results.

Note: An OD value of less than 0.5 absorbance units for the 0 ppb standard may indicate the deterioration of reagents.

Technical support

Not sure if the test works with your specific samples or matrices? Let our longstanding experience in mycotoxin testing work for you. Contact your technical sales representative for more information.

Visit **www.romerlabs.com** to find worldwide contact information.
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