AgraQuant® T-2 Toxin Assay
75/500

Order #: COKAQ6000

Intended Use
The AgraQuant® T-2 Toxin Assay is a direct competitive enzyme-linked immunosorbent assay (ELISA) that determines a quantitative level for the presence of T-2 toxin and is intended for use in grains, cereals, nuts, animal feeds and other commodities.

The AgraQuant® T-2 Toxin Assay has been validated for barley, corn, rice sorghum and wheat.

T-2 Toxin
T-2 toxin is a type A trichothecene. T-2 toxin is produced by fungi of the Fusarium genus, and the most important producer is Fusarium sporotrichioides. This mycotoxin occurs in grains such as wheat, maize, oats, barley, rice, beans and soybeans as well as in some cereal-based products. T-2 toxin inhibits protein synthesis and affects the actively dividing cells such as those lining the gastrointestinal tract, skin, lymphoid and erythroid cells. The effects of T-2 toxin to animals include weight loss or poor weight gain, bloody diarrhea, dermal necrosis or beak lesions, hemorrhage and decreased production (weight gain, eggs, milk, etc.).

Assay Principles
The AgraQuant® T-2 Toxin Assay is a direct competitive enzyme-linked immunosorbent assay (ELISA). T-2 toxin is extracted from a ground sample with 70% methanol. The extract is further diluted at 1:10 using de-ionized or distilled water. The diluted extract and enzyme-conjugated T-2 toxin are mixed in dilution microwells and transferred to the antibody-coated microwells. T-2 toxin in samples and control standards are allowed to compete with enzyme-conjugated T-2 toxin for the antibody binding sites. After a washing step, an enzyme substrate is added and blue color develops. The intensity of the color is inversely proportional to the concentration of T-2 toxin in the sample or standard. A stop solution is then added which changes the color from blue to yellow. The microwells are measured optically using a microwell reader with an absorbance filter of 450nm (OD450). The optical densities of the samples are compared to the OD’s of the standards and an interpretative result is determined.

Precautions
1. Store reagents at 2-8°C (35-46°F) when not in use, and do not use beyond the expiration date.
2. Adhere to incubation times stated in the procedure. Use of incubation times other than those specified may give inaccurate results.
3. The Stop Solution contains acid. Avoid contact with skin or eyes. If exposed, flush with water.
4. Consider all materials, containers and devices that are exposed to the sample or standards to be contaminated with toxin. Wear protective gloves and safety glasses when using the kit.
5. Dispose of all materials, containers and devices appropriately after use.
Procedure

Sample Preparation / Extraction

1. Obtain a representative sample and grind it using a Romer Series II® Mill so that 75% will pass through a 20-mesh screen, 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 100 mL of 70% methanol and seal jar. **Note:** Samples should be extracted in a ratio of 1:5 (w:v) of sample to extraction solution respectively.
4. 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. **Note:** Commodity extracts should have a pH of 6-8. Excessive alkaline or acidic conditions may affect the test results and should be adjusted before testing.
6. Dilute the sample extract 1:10 with deionized or distilled water. For example, add 1 ml of extract to 9 ml of distilled or deionized water.
7. The sample is ready for testing without further preparation.

Assay

**Note:** All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use. It is recommended that an 8-channel pipettor be used to perform the assay. No more than 48 samples and standards total (6 test strips) should be run in one experiment when using an 8-channel pipettor. If an 8-channel pipettor is not used (i.e. using only single channel pipettes), it is recommended that no more than a total of 16 samples and standards (2 test strips) be run in any one experiment.

1. Dilute kit standards (i.e. 0, 75, 150, 300, & 500 ppb) 1:10 with deionized or distilled water in test tubes. For example, add 0.1ml of standard to 0.9 ml of deionized or distilled water and mix.
2. Place the appropriate number of blue-bordered Dilution Strips in a microwell strip holder. One Dilution Well will be required for each standard or sample.
3. Place an equal number of Antibody Coated Microwell strips in a microwell strip holder. Return unused microwell strips to the foil pouch with the desiccant packet and reseal pouch with tape.
4. Measure the required amount of Conjugate from the green-capped bottle (~240 µL/well or 2 mL/strip) and place in a separate container (e.g. reagent boat when using the 8-channel pipettor). Using an 8-channel pipettor, dispense **200 µL of Conjugate** into each blue-bordered Dilution Well.
5. Using a single channel pipettor, add **100 µL of each diluted standard or sample** into the appropriate Dilution Well 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. Using an 8-channel pipettor with fresh tips for each 8-well strip, mix each well by carefully pipetting it up and down 3 times and immediately transfer **100 µL of the contents from each Dilution Well** into a corresponding Antibody Coated Microwell. Incubate at room temperature for 10 minutes. **Note:** Do not agitate the plate to mix as it may cause well-to-well contamination.
6. Empty the contents of the microwell strips into a waste container. Wash by filling each microwell with distilled or deionized water, and then dumping the water 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 wash procedure.
7. Lay several layers of absorbent paper towels on a flat surface and tap microwell strips on towels to expel as much residual water as possible after the fifth wash. Dry the bottom of the microwells with a dry cloth or towel.
8. Measure the required amount of Substrate from the blue-capped bottle (~120 µL/well or 1 mL/strip) and dispense into a separate container (e.g. reagent boat for an 8-channel pipettor). **Pipeette 100 µL of the Substrate** into each microwell strip using an 8-channel pipettor. Incubate at room temperature 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 pipettor). **Pipeette 100 µL of Stop Solution** into each microwell strip using an 8-channel pipettor. The color should change from blue to yellow.
10. Read the strips with a microwell reader using a 450 nm filter. Record OD readings for each microwell.  

**Note:** Air bubbles should be eliminated prior to reading strips as they may affect analytical results.

**Additional Notes:** Ratio of Conjugate to Standard/Sample should remain at 2:1, but volumes of Conjugate and Standards/Samples can be reduced, e.g. using 100µL and 50µL, respectively. The content to be transferred from dilution well to antibody coated well remains the same as 100 µL. Do not return unused reagents to their original bottles. Carefully keep track of the position of Samples and Standards during the assay. Do not mix the assay microwells by shaking at any time during test.

**Interpretation of the Results**

Using either the unmodified OD values or the OD values expressed as a percentage of the OD of the zero (0) standard, construct a dose-response curve using the five standards. Since the amount of T-2 in each standard is known, the unknowns can be measured by interpolation from this standard curve. Results can also be easily calculated using the Romer® Log/Logit spreadsheet that is provided (free of charge) upon request. If the Log/Logit regression model is used for results interpretation, the linearity coefficient \((r^2)\) of the calibration curve should be no less than 0.985. An OD value of less than 0.5 absorbance units for 0ppb standard may indicate deterioration of reagents.

Samples containing less than 75ppb should be reported as "< 75ppb". Samples containing greater than 500ppb should be reported as "> 500ppb". Samples containing greater than 500ppb should be further diluted with deionized or distilled water such that the diluted sample results are in the range of 75-500ppb and reanalyzed to obtain accurate results. The dilution factor must be included when the final result is calculated.

**Performance Characteristics**

- **Limit of detection:** 35 ppb (Determined by the average values of 10 T-2 toxin free samples plus 2 standard deviation).
- **Limit of quantitation:** 75 ppb (Described as the lowest concentration point on the calibration curve that this test can reliably detect T-2 toxin).
- **Range of quantitation:** 75 - 500 ppb (For quantitation of samples above 500ppb samples should be diluted such that the diluted sample results are in a range of quantitation).

**Cross Reactivity**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross reactivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-2</td>
<td>100</td>
</tr>
<tr>
<td>HT-2</td>
<td>44%</td>
</tr>
<tr>
<td>T-2 Triol</td>
<td>1.6%</td>
</tr>
<tr>
<td>T-2 Tetraol</td>
<td>&lt;0.04%</td>
</tr>
<tr>
<td>Verrucarol</td>
<td>&lt;0.04%</td>
</tr>
</tbody>
</table>

**Materials Supplied With Kit**

- 96 antibody coated microwells (12 eight-well strips) in a microwell holder (sealed in a zip-lock foil pouch)
- 96 non-coated dilution microwells (12 eight-well strips marked with blue at base)
- 5 vials of 1.5mL of each T-2 toxin standard. Standard concentrations are 0, 75, 150, 300 and 500 ppb, respectively. Standards need further dilution of 1:10 with deionized or distilled water before assay.
- 1 bottle of 25mL of T-2 toxin conjugate (green-capped bottle)
- 1 bottle of 15mL of substrate solution (blue-capped bottle)
- 1 bottle of 15mL of stop solution (red-capped bottle)

**Materials Required But Not Provided With Kit**

**Extraction Procedure**

- *EQMMS2010: Romer Series II® Mill or equivalent*
- *EQOLE1025: Blender or a tightly sealing jar with lid*
- *EQOLE1010: Balance, 400 g*
- *EQOLE1050: Graduated cylinder: 100mL*
• Container with a minimum 125mL capacity
• *Whatman#1 filter paper, or equivalent
• *Filter funnel

Assay Procedure
• *8-channel and single channel pipettors capable of pipetting 100µL and 200µL with tips
• *EQOLE1300: Timer
• *COKAD1150: Wash bottle
• Distilled or de-ionized water
• Absorbent paper towels
• *3 reagent boats for use as reagent containers for an 8-channel pipettor
• *Microwell reader with a 450nm filter

*Items available from Romer Labs, Inc.® - Americas Division

For further information please contact:
Technical Services
Romer Labs Singapore Pte. Ltd.
3791 Jalan Bukit Merah #08-08
e-Centre@redhill, Singapore, 159471
Tel: (65) 62755432
Fax: (65) 62755584
Web: http://www.romerlabs.com
Email: salesasia@romerlabs.com

Warranty
The user assumes all risk in using Romer Labs, Inc.® products and services. Romer Labs, Inc.® will warrant that its products and services meet all quality control standards set by Romer Labs, Inc.®, and Romer Labs, Inc.® will, at its option, repair or replace any product, components, or repeat services which prove to be defective in workmanship or material within product specific warranty periods or expiration dates and which our examination shall disclose to our satisfaction to be defective as such. This warranty is expressly in lieu of all other warranties, expressed or implied, as to description, quality, merchantability, fitness for any particular purpose, productiveness, or any other matter. Romer Labs, Inc.® shall be in no way responsible for the proper use of its products. Romer Labs, Inc.® hereby disclaims all other remedies, warranties, guarantees or liabilities, expressed or implied, arising by law or otherwise, and it shall have no liability for any lost profits or damage, direct, indirect or otherwise, to person or property, in connection with the use of any of its products or services. This warranty shall not be extended, altered or varied except by a written instrument signed by an authorized representative of Romer Labs, Inc.®