RIDASCREEN® T-2 Toxin

Enzymimmunoassay zur quantitativen Bestimmung von T-2 Toxin

Enzyme immunoassay for the quantitative analysis of T-2 toxin

Art. No.: R3801

In vitro Test
Lagerung bei 2 - 8 °C
Storage at 2 - 8 °C

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RIDASCREEN® T-2 Toxin

Brief information

RIDASCREEN® T-2 Toxin (Art. No.: R3801) is a competitive enzyme immunoassay for the quantitative analysis of T-2 toxin in cereals and feed. All reagents required for the enzyme immunoassay - including standards - are contained in the test kit. The test kit is sufficient for 96 determinations (including standards). A microtiter plate spectrophotometer is required for quantification.

Sample preparation: extraction, filtration and dilution

Time requirement: sample preparation (for 10 samples) approx. 30 min
Test implementation (incubation time) approx. 1 h 30 min

Detection limit: < 5 ppb (approx. 3.5 µg/kg)

Recovery rate: in spiked cereal samples approx. 90 % ± 10 %

Specificity: The specificity of the RIDASCREEN® T-2 Toxin test was established by analyzing the cross-reactivity to corresponding mycotoxins.

- T-2 toxin: 100 %
- Acetyl T-2 toxin: approx. 114 %
- HT-2 toxin: approx. 7 %
- Iso T-2 toxin: approx. 2 %
1. Intended use

RIDASCREEN® T-2 Toxin is a competitive enzyme immunoassay for the quantitative analysis of T-2 toxin in cereals and feed.

2. General

T-2 toxin belongs to the trichothecene group of mycotoxins and is formed by fungi of the genus Fusarium. T-2 toxin is often found in agricultural commodities, although the incidence and the concentrations found show a broad regional variation. Due to its cytotoxic and immunosuppressive mode of action T-2 toxin is a threat for human and animal health.

3. Test principle

The basis of the test is the antigen-antibody reaction. The microtiter wells are coated with capture antibodies directed against anti-T-2 toxin antibodies. T-2 toxin standards or sample solutions, T-2 toxin enzyme conjugate and anti-T-2 toxin antibodies are added. Free T-2 toxin and T-2 toxin enzyme conjugate compete for the T-2 toxin antibody binding sites (competitive enzyme immunoassay). At the same time, the anti-T-2 toxin antibodies are also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. Enzyme substrate and chromogen are added to the wells and incubated. Bound enzyme conjugate converts the chromogen into a blue product. The addition of the stop solution leads to a color change from blue to yellow. The measurement is made photometrically at 450 nm. The absorbance is inversely proportional to the T-2 toxin concentration in the sample.
4. Reagents provided

Each kit contains sufficient materials for 96 measurements (including standard analyses). Each test kit contains:

1 x Microtiter plate (12 strips with 8 removable wells each)  
   coated with capture antibodies
6 x Standard solutions, (1.3 ml each)  
   0 ppb (zero standard), 0.1 ppb, 0.2 ppb, 0.4 ppb, 0.8 ppb, 1.6 ppb  
   T-2 toxin in aqueous solution, ready to use
1 x Conjugate (5 ml) ..................................................................................red cap  
   peroxidase conjugated T-2 toxin  
   ready to use
1 x Anti-T-2 toxin antibody (5 ml) ......................................................... black cap  
   monoclonal, ready to use
1 x Substrate (7 ml) ...............................................................................green cap  
   contains urea peroxide
1 x Chromogen (7 ml) .............................................................................. blue cap  
   contains tetramethylbenzidine
1 x Stop solution (14 ml) ...........................................................................yellow cap  
   contains 1 N sulfuric acid
1 x Sample dilution buffer (50 ml)

5. Materials required but not provided

5.1. Equipment:

−microtiter plate spectrophotometer (450 nm)  
−grinder (mill)  
−magnetic stirrer  
−paper filter or centrifuge  
−variable 20 µl - 200 µl and 200 - 1000 µl micropipettes
5.2. Reagents:

- methanol
- for dilution of samples (for dilutions > dilution factor of 35):
  PBS buffer, pH 7.2: (0.55 g NaH$_2$PO$_4$ x H$_2$O + 2.85 g Na$_2$HPO$_4$ x 2 H$_2$O + 9 g NaCl; fill up to 1000 ml with methanol/distilled water 10/90 (v/v)

This buffer has to contain 10 % methanol in order to keep a 10 % methanol concentration of the samples used in the assay (also see section 9.1.).

6. Warnings and precautions for the users

The standards contain T-2 toxin, particular care should be taken. Avoid contact of the reagent with the skin (use gloves).

Decontamination of the glassware and T-2 toxin solutions is best carried out using a sodium hypochlorite (bleach) solution (10 % (v/v)) overnight (adjust solution with HCl to pH 7).

The stop solution contains 1 N sulfuric acid (R36/38, S2-26).

7. Storage instructions

Store the kit at 2 - 8 °C (35 - 46 °F). Do not freeze any test kit components.

Return any unused microwells to their original foil bag, reseal them together with the desiccant provided and further store at 2 - 8 °C (35 - 46 °F).

T-2 toxin is light sensitive, therefore, avoid exposure to direct light.

The colorless chromogen solution is light sensitive, therefore, avoid exposure to direct light.

No quality guarantee is accepted after the expiration date on the kit label.

Do not interchange individual reagents between kits of different lot numbers.

8. Indication of instability or deterioration of reagents

- any bluish coloration of the chromogen solution prior to test implementation
- a value of less than 0.6 absorbance units ($A_{450\text{ nm}} < 0.6$) for the zero standard
9. Preparation of Samples

The samples should be stored in a cool place, protected against light.

A representative sample (according to accepted sampling techniques) should be ground and thoroughly mixed prior to proceeding with the extraction procedure.

9.1. Cereals and feed

- weigh 5 g of sample and dissolve with 25 ml of methanol/distilled water 70/30 (v/v) for extraction *)
- stir the extract for 10 min on a magnetic stirrer
- filter the extract over paper filter or centrifuge the extract
- dilute the filtrate or the supernatant 1:7 (1+6) with sample dilution buffer (see 4.), e.g. 50 µl of filtrate or supernatant + 300 µl of sample dilution buffer
- use 50 µl per well in the assay (if T-2 toxin concentrations less than 56 ppb are expected)

*) sample size may be increased if required, but the volume of methanol/distilled water 70/30 (v/v) must be adapted accordingly, e.g.: 25 g in 125 ml methanol/distilled water 70/30 (v/v) or 50 g in 250 ml methanol/distilled water 70/30 (v/v)

Remark:

At high T-2 toxin concentrations (> 56 ppb) the 1:7 diluted extract solution must be further diluted e.g. 1:10 (1+9) with PBS buffer containing 10 % methanol (see 5.2.), e.g. 50 µl of the diluted extract solution + 450 µl PBS buffer with 10 % methanol.
10. Test implementation

10.1. Preliminary comments

Bring all reagents to room temperature (20 - 25 °C / 68 - 77 °F) before use.

10.2. Test procedure

Carefully follow the recommended washing procedure. Do not allow microwells to dry between working steps.

1. Insert a sufficient number of wells into the microwell holder for all standards and samples to be run in duplicate. Record standard and sample positions.
2. Add 50 µl of standard or prepared sample to separate duplicate wells; use a new pipette tip for each standard or sample.
3. Add 50 µl of enzyme conjugate (red cap) to the bottom of each well.
4. Add 50 µl of the anti-T-2 toxin antibody (black cap) to each well. Mix gently by shaking the plate manually and incubate for 1 h at room temperature (20 - 25 °C / 68 - 77 °F).
5. Pour the liquid out of the wells and tap the microwell holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. Fill all the wells with 250 µl distilled water and pour out the liquid again. Repeat the washing procedure two times.
6. Add 50 µl of substrate and 50 µl of chromogen to each well. Mix gently by shaking the plate manually and incubate for 30 min at room temperature (20 - 25 °C / 68 - 77 °F) in the dark.
7. Add 100 µl of the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450 nm. Read within 30 minutes after addition of stop solution.

11. Results

A special software, the RIDA®SOFT Win (Art. No. Z9999), is available for evaluation of the RIDASCREEN® enzyme immunoassays.

The course of the standard curve is shown in the Quality Assurance Certificate enclosed in the test kit.
Remark for the calculation without software:

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\frac{\text{absorbance standard (or sample)}}{\text{absorbance zero standard}} \times 100 = \% \text{ absorbance}
\]

The zero standard is thus made equal to 100 % and the absorbance values are quoted in percentages. The values calculated for the standards are entered in a system of coordinates on semilogarithmic graph paper against the T-2 toxin concentration [µg/kg].

In order to obtain the T-2 toxin concentration in µg/kg actually contained in a sample, the concentration read from the calibration curve must be further multiplied by the corresponding dilution factor. When working in accordance with the regulation stated, the dilution factors are as follows:

- cereals, feed: 35 (or 350)

Therefore, the standard curve is in the range of 3.5 to 56 µg/kg (ppb) (or in the range of 35 to 560 µg/kg (ppb)) T-2 toxin for cereal or feed samples.

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