RIDASCREEN® T-2 / HT-2 Toxin

REF R3805

Enzymimmunoassay zur quantitativen Bestimmung
von T-2 und HT-2 Toxin

Enzyme immunoassay for the quantitative determination
of T-2 and HT-2 Toxin

In vitro Test

Lagerung bei 2 - 8 °C
Storage at 2 - 8 °C

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RIDASCREEN® T-2 / HT-2 Toxin

Brief information

RIDASCREEN® T-2 / HT-2 Toxin (Art. No.: R3805) is a competitive enzyme immunoassay for the quantitative analysis of T-2 and HT-2 toxin in oats, barley, corn and wheat.
All reagents required for the enzyme immunoassay, including standards, are contained in the test kit. The test kit is sufficient for a maximum of 96 determinations (including standards). A microtiter plate spectrophotometer is required for quantification.

Sample preparation:	extraction, centrifugation and dilution
Time requirement:	sample preparation (for 10 samples)……ca. 30 min
	test run (incubation time) ……..…45 min
Measuring range:	10 - 360 µg/kg
Limit of detection LoD:	Oats…….13 ppb (13 µg/kg)
(depending on matrix)	Barley……11 ppb (11 µg/kg)
	Corn……...10 ppb (10 µg/kg)
	Wheat……14 ppb (14 µg/kg)
Limit of Quantification	Oats…….24 ppb (24 µg/kg)
LoQ:	Barley……15 ppb (15 µg/kg)
	Corn……...19 ppb (19 µg/kg)
	Wheat……...33 ppb (33 µg/kg)
Recovery rate:	Trilogy® Reference material (Wheat, Corn).mean 96%
	spiked wheat-, barley-, corn samples……...mean 79%
	spiked oat samples..……. 69%
	The RIDASCREEN® T-2 / HT-2 Toxin test detects T-2 / HT-2 toxin in corn, oats, wheat, and barley samples. The recovery of wheat and corn was validated with naturally contaminated and spiked samples. Oats and barley were only validated with spiked samples.
Specificity:	Cross-reactivity to T-2 Toxin……...…….72%

The specificity of the RIDASCREEN® T-2 / HT-2 test was determined by analyzing the cross-reactivities to corresponding substances in buffer system. In samples, the specificity may deviate from those determined in the buffer system due to matrix effects. Prior to the analysis of cross-reactive substances, the user has to determine the Limit of Detection (LoD) and the Recovery for the substance in the respective sample matrix. The test cannot discriminate between analytes and cross-reactive substances.

In order to increase the quality of assessment when performing ELISA procedures, we refer additionally to our Good ELISA Practice brochure. It lists minimum standards and conditions that are required when using test kits of R-Biopharm AG to perform ELISA analysis. The brochure can be retrieved, printed, and downloaded from the website https://food.r-biopharm.com/media/technical-guides/.

Note:

If the validation was carried out with spiked samples, naturally contaminated samples of different grain varieties can lead to deviating results.

Related products

RIDA® QUICK T-2 / HT-2 Toxin RQS ECO (R5304)
RIDASCREEN® FAST T-2 Toxin (R5302)
RIDASCREEN® T-2 Toxin (R3801)
PuriTox Trichothecene (TC-T220)
EASI-EXTRAKT® T-2 / HT-2 (RBRP43; RBRP43B)
DZT MS-PREP® (RBRP73/RBRP73B)
Trilogy® Dried Standard T-2 Toxin (TAS-M24DA1-5)
Trilogy® Liquid Standard T-2 Toxin (TAS-M24LA1-5)
Trilogy® Dried Standard HT-2 Toxin (TAS-M25DA1-5)
Trilogy® Liquid Standard HT-2 Toxin (TAS-M25LA1-5)

1. Intended use

RIDASCREEN® T-2 / HT-2 Toxin is a competitive enzyme immunoassay for the quantitative analysis of T-2 and HT-2 toxin in oats, corn, barley, and wheat.

2. General

T-2 and HT-2 toxin belong to the trichothecene group of mycotoxins and are formed by fungi of the genus *Fusarium*. T-2 and HT-2 toxin are often found in agricultural commodities, although the incidence and the concentrations found show a broad regional variation. Due to their high cytotoxic and immunosuppressive mode of action, T-2 / HT-2 toxins are 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 / HT-2 toxin antibodies. Standards or sample solutions, T-2 toxin enzyme conjugate and anti-T-2 / HT-2 toxin antibodies are added. Free T-2 / HT-2 toxin and T-2 toxin enzyme conjugate compete for the T-2 / HT-2 toxin antibody binding sites (competitive enzyme immunoassay). At the same time, the anti-T-2 / HT-2 toxin antibodies are also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. Substrate/Chromogen is added to the wells, 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 / HT-2 toxin concentration in the sample.

4. Reagents provided

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

Component	Cap color	Format		Volume
Microtiter plate M	-	Ready to use		96 wells
2x Extractor buffer oats	White	Concentrate	10x	120 mL each
Standard 1	White	Ready to use	0 µg/L	1.3 mL
Standard 2	White	Ready to use	1 µg/L	1.3 mL
Standard 3	White	Ready to use	3 µg/L	1.3 mL
Standard 4	White	Ready to use	6 µg/L	1.3 mL
Standard 5	White	Ready to use	12 µg/L	1.3 mL
Standard 6	White	Ready to use	36 µg/L	1.3 mL
Wash buffer salt		Dissolve the salt		
Conjugate	Red	Ready to use		6 mL
Antibody	Black	Ready to use		6 mL
Substrate/Chromogen Red Chromogen Pro	Brown	Ready to use		10 mL
Stop Solution	Yellow	Ready to use		14 mL

5. Materials required but not provided

5.1. Equipment:

- Gloves
- Scale (measurement range at least up to 50 g and precision of ± 0.01 g)
- Laboratory mincer / grinder, mortar, ultra-turrax or homogenizer
- Microtiter plate spectrophotometer (450 nm)
- grinder (mill)
- Centrifuge
- Variable 20 - 200 µL and 100 - 1000 µL micropipettes
- Optional: RIDASOFT® Win.NET Food & Feed (Art. No. Z9996FF)
- Optional: Multistepper or 8-channel pipette for 50,100 and 250 µL

5.2. Reagents:

- Methanol (70 %)
- Methanol (35 %) for further dilution of high contaminated samples
- Distilled or deionized water

6. Warnings and precautions for the users

The product / test is only suitable within the scope of its intended use.

This test should only be carried out by trained laboratory personnel. The instructions for use must be strictly followed.

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

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

This kit may contain hazardous substances. For hazard notes on the contained substances please refer to the appropriate safety data sheets (SDS) for this product, available online at www.r-biopharm.com.

Do not reuse wells of the microtiter strips (coated microtiter plate). Use separate pipette tips for each standard and each sample extract to avoid cross contamination.

All reagents and materials must be recovered or disposed after use at customers own responsibility according to the protection of human health and the environment. Please observe the applicable national regulations concerning waste disposal (e.g. Waste Management Act, Regulations on Dangerous Chemicals, etc.).

7. Storage instructions

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

To avoid moisture inside the wells, open the foil bag for withdrawal of microwells only after having reached room temperature (20 - 25 °C / 68 - 77 °F).

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).

HT-2 toxin is light sensitive; therefore, avoid exposure of standards to direct light.

The reddish substrate/chromogen is light sensitive, therefore, avoid exposure to direct light.

Do not use the test kit after the expiration date (see test 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 reddish substrate/chromogen prior to test implementation
- A value of less than 0.6 absorbance units ($A_{450\ nm} < 0.6$) for the zero standard

9. Sample preparation

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

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

9.1. Oats

For preparation of oat samples, a special extraction buffer is needed. Please use the extraction buffer oats concentrate contained in the kit and dilute it 1:10 (e.g. 10 mL + 90 mL distilled water) to obtain ready-to-use extraction buffer oats. Ready-to-use extraction buffer oats should be stored at 2 - 8 °C (36 - 46 °F) and expires after approx. 8 - 10 weeks.

- Weigh out 5 g of ground sample and add 25 mL of ready-to-use extraction buffer oats *)
- Shake the sample for 10 min (overhead)
- Centrifuge: 10 min / 3000 g / at room temperature (20 - 25 °C / 68 - 77 °F)
- Dilute the supernatant 1:2 (1+1) with methanol/distilled water (70/30; v/v), (e. g. 1 mL supernatant + 1 ml of methanol/distilled water (70/30; v/v))
- Use 50 µL per well in the assay

*) sample size may be increased if required, but the volume of ready-to use extraction buffer oats must be adapted accordingly, e.g.: 25 g in 125 mL ready-to-use extraction buffer oats or 50 g in 250 mL ready-to-use extraction buffer oats. Buffer contained in the kit will then be sufficient for a lower number of oat samples, respectively.

9.2. Grains (barley, corn, wheat)

- Weigh out 5 g of ground sample and dissolve with 25 mL of methanol/distilled water (70/30; v/v) for extraction *)
- Shake the extract for 10 min (overhead)
- Centrifuge: 10 min / 3000 g / at room temperature (20 - 25 °C / 68 - 77 °F)
- Dilute the supernatant 1:2 (1+1) with dest. water
  (e. g. 1 mL supernatant + 1 ml of dest. water)
- use 50 µL per well in the assay

*) 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 / HT-2 toxin concentrations (> 360 ppb) the 1:2 diluted extract solution must be further diluted (e.g. 1:10 (1+9) with methanol (35 %), means 50 µL of the diluted extract solution + 450 µL methanol (35 %)). The given example would result in an additional dilution factor of 10.

10. Test implementation

10.1. Preliminary comments

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

A PBS-Tween buffer is needed as **wash buffer**; please use the wash buffer salt contained in the kit (see 4.). Dissolve the total content of the pouch in one liter of distilled water. The ready to use washing buffer expires after approx. 4 - 6 weeks, stored at 2 - 8 °C (36 - 46 °F).

Alternatively: Dissolve the contents of the pouch in 100 mL of distilled water to obtain a 10fold concentrated wash buffer. This 10fold concentrate expires after approx. 8 - 12 weeks, stored at room temperature (20 - 25 °C / 68 - 77 °F). Use one part of this

concentrate and dissolve with 9 parts of distilled water to obtain the ready to use wash buffer.

Components should be stored immediately at 2 - 8 °C (35 - 46 °F) when no longer required.

10.2. Test procedure

Carefully follow the recommended washing procedure to obtain unambiguous results. Do not allow microwells to dry between work 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 conjugate to the bottom of each well.
4. Add 50 µL of antibody to each well. Mix gently by shaking the plate manually and incubate for 30 min 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 wash buffer (see 10.1.) and pour out the liquid again. Repeat two more times.
6. Add 100 µL of substrate/chromogen to each well. Mix gently by shaking the plate manually and incubate for 15 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 min after addition of stop solution.

11. Evaluation

A special software, the **RIDASOFT® Win.NET Food & Feed (Art. No. Z9996FF)** is available for evaluation of the RIDASCREEN® enzyme immunoassays.

For the evaluation it should be clarified, that all quality criteria are fulfilled for the current test run. The course of the standard curve is shown in the certificate of analysis (CoA) enclosed in the test kit.

Remark for the calculation without software:

$$\frac{\text{absorbance standard (or sample)}}{\text{absorbance zero standard}} \times 100 = B/B_0\ (\%)$$

The zero standard is thus equated 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 / HT-2 toxin concentration [µg/L].

12. Result interpretation

In order to obtain the T-2 and HT-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:

Oats, corn, barley, wheat.....10 (or 100*)

* see 9. sample preparation, remark

Therefore, the measurement range of the test is 10 to 360 µg/kg (ppb) T-2 / HT-2 toxin for oats and grain (or in the range of 100 to 3600 µg/kg (ppb)).

Results between LoD and LoQ may indicate a low T-2 / HT-2 concentration in the sample. Therefore, such results should not be reported with a quantitative value, but qualitative as “< LoQ”.

A result below the LoD does not exclude an T-2 / HT-2 contamination below the detection limit of the assay. The result should be reported accordingly.

A further dilution of samples is recommended for absorbance values ($A_{450\ nm}$) > standard 6. In case of a further dilution, the additional dilution factor must be taken into account when calculating the T-2 / HT-2 concentration.

Compared to the certificate, higher absorbance values ($A_{450\ nm}$) for the standard curve, especially for the zero standard, may be a result of insufficient washing or T-2 / HT-2 contamination.

13. Limits of the method

Test results may vary depending on the sample matrix, the actual test procedure, and the laboratory environment.

Detection limits (LoD) and quantification limits (LoQ) depend on the respective sample matrix, the degree of processing, and the extraction method.

14. Recommendation

In order to ensure a high analytical performance we recommend to analyze each sample material in duplicates. Each laboratory may decide to perform the test in single determinations after a qualified risk management analysis. This has no influence on the function of the test kit. However, it should be noted that this increases the risk of overlooking errors in the performance of the test (e.g. pipetting errors). Moreover, a higher result variation will occur when pipetting in single determinations.

In order to ensure a high analytical performance we recommend:

- Pre-flush pipette tips with standard or sample extract prior to pipetting.
- Carry along test controls for quality control. Toxin free and toxin containing (spiked) samples should be used.
- In case of extremely acidic or basic samples, adjust the sample's pH value (pH 6.5 - 7.5) to neutral prior to extraction.
- To do spike experiments to ensure an accurate and correct test procedure. An example of a spiking experiment is given in the validation report.
- To contact sales@r-biopharm.de if automates (e.g. ThunderBolt® / Bolt™) are used.

15. Further Application notes

Further application notes are available on request.

For further product information and applications, please contact your local distributor or R-Biopharm at this address: sales@r-biopharm.de.

Version overview

Version number	Chapter and title
2015-09-09	Release version
2024-01-30	Current version General revision Changes made: – Brief information – Equipment – Chapter 12-15. are added

Explanation of symbols

General symbols:

 Follow the instructions for use

 Batch number

 Expiry date (YYYY-MM-DD)

 Storage temperature

 Article number

 Number of test determinations

 Manufacturing date (YYYY-MM-DD)

 Manufacturer + address

Disclaimer

The user assumes all risk in using R-Biopharm AG´s products and services.

R-Biopharm AG will warrant that its products and services meet all quality control standards set by R-Biopharm AG, and R-Biopharm AG will, at its option, replace or repair any components, product 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 quality, description, fitness for any particular purpose, merchantability, productiveness, or any other matter. R-Biopharm AG shall be in no way responsible for the proper use of its products and 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 R-Biopharm AG.

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