Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

AccuDiag™ T-2 Toxin ELISA Kit

Cat# 5149-8

Test	T-2 Toxin ELISA
Recovery (spiked samples)	93-115%
Total Time	~ 20 min.
Shelf Life	12-14 Months from the manufacturing date
Specificity	N/A
Sensitivity	0.9-117 ppb

INTENDED USE

T-2 Toxin in addition to deoxynivalenol, zearalenone, the fumonisines and other trichothecenes belongs to the fusarium toxins. These toxins are already produced on the field in consequence of a contact of the cereals by fusarium species. Acute toxic dosages can result in gastroenteritis, damage of bone marrow up to necroses of skin and respiratory passages.

T-2 toxin has a high stability against temperature and can therefore also be detected in bakery products. In Russia the legislator set limit values for T-2 Toxin in food between 50 and 100 ppb. The introduction of limit values in the European union is discussed since many years. Thus an observation of food and feed with respect to the concentration of T-2 Toxin is increasingly obligatory.

The **Diagnostic Automation, Inc. T-2 Toxin ELISA** represents a highly sensitive detection system and is particularly capable of the quantification of T-2 Toxin contaminations in cereals, beer, milk and meat.

Due to the cross-reactivity to HT-2 Toxin the test is also suitable for the detection of this mycotoxin to a certain extent.

TEST PRINCIPLE

The Diagnostic Automation, Inc. **T-2 Toxin** quantitative test is based on the principle of the enzyme-linked immunosorbent assay. An antibody binding protein is coated on the surface of a microtiter plate. The standards and samples respectively are pipetted together with a T-2 Toxin-peroxidase conjugate and a rabbit-anti-T-2 Toxin antibody into the appropriate wells. The conjugate competes with the T-2 Toxin of samples/standards for the limited number of antibody sites. Simultaneously the anti-T-2 Toxin antibody is bound to the antibody-binding protein coated on the microtiter plate. After 10 min incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated for 10 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of T-2 Toxin is indirectly proportional to the colour intensity of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1. **Microtiter plate** consisting of 12 strips with 8 breakable wells each, coated with antibody-binding protein.
2. **T-2 Toxin Standards (0, 0.5, 2.5, 10, 25, 50 ng/mL):** 6 vials with 1 mL each, dyed red, ready-to-use.
3. **Anti-T-2 Toxin Antibody (rabbit):** 6 mL, dyed blue, ready-to-use.
4. **Conjugate (T-2 Toxin-Peroxidase):** 6 mL, dyed red, ready-to-use.
5. **Substrate Solution (TMB):** 15 mL, ready-to-use.
6. **Stop Solution (0.5 M H_2SO_4):** 15 mL; ready-to-use.
7. **Sample Diluent (PBS):** 2 x 60 mL dyed red, ready-to-use.
8. **Washing Solution (PBS + Tween 20):** 60 mL as 10x concentrate. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.
9. Plastic bag to store unused microtiter strips.
10. Instruction Manual.

Materials required but not provided

1. 50, 100 and 1000 µL-micropipettes
2. ELISA reader (450 nm)
3. Centrifuge
4. Ultra-Turrax, mixer, vortex

Reagents

1. Double distilled water
2. Methanol

Sample Preparation

Cereals / Meat

1. Suspend 5 g of previously ground sample in 25 mL of 60% methanol.
2. Mix suspension for 5 minutes.
3. Centrifuge extract at 3000 g for 10 minutes.
4. Dilute 100 µL of supernatant with 500 µL of sample diluent and test the sample in the ELISA.
5. Sample dilution factor: F=30.

Beer / Milk

1. Add 10% of methanol to the sample diluent.
2. Carbonized beer samples should be previously degassed by moderate heating.
3. Cloudy beers (such as beer brewed from wheat) / gyle should previously be sterile-filtered.
4. Degrease whole milk samples by centrifugation
5. Dilute 100 µL beer / milk with 900 µL sample diluent. Containing 10% methanol
6. Sample dilution factor: F=10.

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Due to the linearity of the test system further dilution of high concentrated samples is generally possible. In all cases further dilution should be done with sample diluents containing 10% of methanol.

ASSAY PROCEDURE

1. Prepare samples as described above.
2. Pipette 100 µL standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 50 µL T-2 Toxin-peroxidase conjugate and 50 µL anti-T-2 Toxin antibody into each well (consider sequence!).
3. Incubate for 10 minutes at room temperature.
4. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipette 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.
5. Pipette 100 µL of substrate solution into each well.
6. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 10 minutes at room temperature.
7. Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue colour will turn yellow upon addition.
8. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The color is stable for 30 minutes.

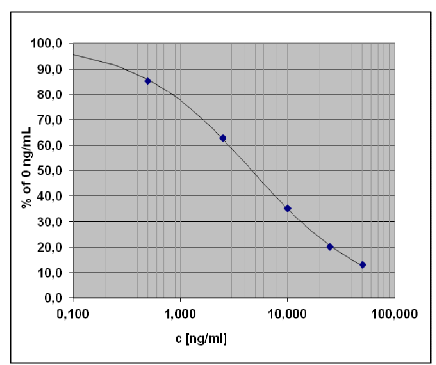
RESULTS

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ng/mL on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis.
3. Using the mean optical density value for each sample, determine the corresponding concentration of T-2 toxin in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. The diluted samples must be further converted by the appropriate **sample dilution factor**. The factors are listed for each sample matrix in the *sample preparation* section.

Typical Standard Values

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ng/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

T-2 Toxin (ng/mL)	(% binding of 0 ng/mL)
0	100
0.5	85
2.5	62
10	35
25	20
50	13



PERFORMANCE CHARACTERISTICS

Sensitivity

The limit of detection (LOD) of the **Diagnostic Automation, Inc. T-2 Toxin test** is 0.3 ng/mL.

Validation experiments with common matrices resulted in the following LODs [ppb].

Matrix	LOD
Wheat	8.7
Rye	11.1
Barley	8.0
Oats	11.7
Corn	9.9
Rice	1.8
Meat (pork)	0.9
Milk	3.5
Beer	2.8

The limit of quantification (LOQ) of the Diagnostic Automation, Inc. **T-2 Toxin test** is 0.5 ng/mL.

Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

Recovery

Matrix	Recovery
Rye flour	93%
Corn flour	101%
Rice flour	115%
Meat (pork)	101%
Milk	96%
Beer	97 %

Linearity

The serial dilution of spiked samples (rye flour, corn flour, rice flour, meat, milk and beer) resulted in a dilution linearity of 86-107%.

Precision

Precision	Value
Intra-assay Precision	3-8%
Inter-assay Precision	9-11%

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Cross-reactivity relative to T-2 Toxin (=100%)

HT-2 Toxin	3.0 %
T-2 Triol	0.35%
T-2 Tetraol	0.07%

PRECAUTION

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

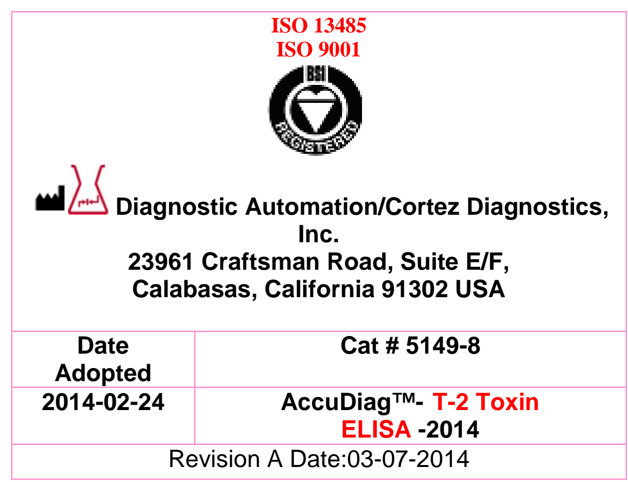
1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipette, ELISA reader etc.)

Health and safety instructions

1. Do not smoke or eat or drink or pipette by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

REFERENCES

1. Schwake-Anduschus C, et al. (2010) – Occurrence of Fusarium T-2 and HT-2 toxins in oats from cultivar studies in Germany and degradation of the toxins during grain cleaning treatment and food processing. Food Addit Contam, 27(9):1253-60
2. Kankkunen P, et al. (2009) – Trichothecene mycotoxins activate inflammatory response in human macrophages. J Immunol, 182(10):6418-25
3. Yoshizawa T, et al. (2004) – A practical method for measuring Deoxynivalenol, Nivalenol, and T-2 + HT-2 Toxin in foods by an enzyme-linke immunosorbent assay using monoclonal antibodies. Biosc Biot Biochem, 68(10):2076-85
4. Chu F S, et al. (1986) – Improved method for production of antibodies against t-2 toxin and diacetoxyscirpenol in rabbits. Appl Env Microb, 51(1):132-37
5. Ohtani K, et al. (1988) – Improved preparation of T-2 toxin-protein conjugates. Toxicon, 26(11):1107-11
6. Katja Bernhardine (2008) – Entwicklung und Validierung von Enzymimmuntests zum Nachweis von T-2 Toxin und HT-2 Toxin sowie Vorkommen dieser Mykotoxine in Lebensmitteln des deutschen Marktes. Dissertation, Tierärztliche Fakultät München
7. Suproniene S, et al. (2010) – Distribution of trichothecene and zearalenone producing fusarium species in grain of different cereal species and cultivars grown under organic farming conditions in Lithuania. Ann Agric Environ Med, 17:79-86
8. Barthel J, et al. (2012) – Occurrence of type A, B and D trichothecenes in barley and barley products from the Bavarian market. Mycotoxin Res, 28(2)_97-106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Date Adopted	Cat # 5149-8
2014-02-24	AccuDiag™- T-2 Toxin ELISA -2014
Revision A Date:03-07-2014	