T2 Toxin ELISA kit

Enzyme immunoassay for the detection of T2 Toxin

(code TT200)

Kit ELISA for T2 TOXIN (code TT200) is an ELISA assay prepared for an immunoenzymatic assay for the quantitative analysis of toxin T2.
The kit contains the procedure and the materials sufficient for 96 determinations including standards.
A microtiter plate photometer or a strip photometer is required.

Analysable samples
Cereals.

Sample preparation
Grinding, mixing, extraction in methanol-water, filtration, dilution.

Assay time: 15 minutes (sample preparation not included).

Detection limit
25 ppb.

Specificity	
Compound	**Cross-reactivity %**
T2	100
H T2	38
H T2 triol	1.6
H T2 tetraol	< 0.04
Verrucarol	< 0.04

1 TEST PRINCIPLES

The basis of the test is the antigen-antibody reaction. Polystirene microtiter wells are coated with purified rabbit antibody directed against sheep IgG. Anti-T2 toxin antibody, T2 toxin-enzyme conjugate and T2 toxin standards or samples are added. After the incubation, microtiter wells are washed to remove unbound enzyme conjugate. The chromogenic substrate is added to the wells. The enzyme converts the colourless chromogen into a coloured (blue) product. The addition of the stop solution transforms the blue colour into a yellow compound. The absorbance is measured by a microplate reader at 450 nm. The colour intensity is inversely proportional to the T2 toxin concentration in the standard or in the sample.

2 PROVIDED REAGENTS

Microtiter plate: 96 wells (12 strips of 8 wells) coated with anti-rabbit IgG., in an aluminium bag with a desiccant bag.
As the strips are breakable, the wells can be used individually. For this purpose, it is sufficient to get out the wells from the sheath and to break the joint.
T2 Toxin std: 5 amber glass vials containing 2 ml of standard equivalent to the following amounts of T2 Toxin in the sample: 0, 25, 75, 200, 500 ppb.
Enzyme Conjugate: 1 plastic vial containing 10 ml.
Anti T2-toxin antibody: 1 plastic vial containing 10 ml.
Developing solution: 1 amber plastic vial containing 14 ml.
Stop solution: 1 plastic vial containing 14 ml.

3 REQUIRED BUT NON PROVIDED MATERIALS

- Deionized or distilled water.
- Methanol.

Equipment
- Analytical grinder.
- Balance.
- 100 ml or more graduated cylinder.
- Container of 100 ml for the extraction of the sample
- Filter paper (Whatman 1).
- Microtubes.
- 20 – 200 µl, 200 – 1000 µl micropipette with tips.
- 50 – 300 µl multichannel micropipette with tips.
- Blotting paper.
- Microtiter plate or strip reader equipped with a 450 nm filter.

4 WARNINGS AND PRECAUTIONS FOR THE USERS

- For *in vitro* diagnostic use only.
- Some reagents contain preservative. The standard solutions are toxic because of methanol. The presence of T2 toxin in the solution does not make them more dangerous because the concentration is very low. The stop solution contains hydrochloric acid and is corrosive.
- Handle the reagents with caution, avoiding contact with skin, eyes and mucous membranes.
- Safety data sheet available on Tecna's web site.

5 HANDLING AND STORAGE INSTRUCTIONS

- Store the kit at +2/+8 °C and do not freeze components.
- Reseal the unused strips of the microtiter plate in the bag together with the desiccant bag provided.
- Do not use components after the expiration date.
- Do not intermix components between different kit lots.
- Do not use photocopies of the instruction booklet. Keep always to the instruction booklet that is included inside the kit.

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6 SAMPLES PREPARATION

- Finely grind the sample and mix it to make it homogeneous.
- Weigh 20 g of the sample and add 100 ml of a solution of 70% methanol in deionized or distilled water.
- Blend or shake thoroughly for 3 minutes.
- Let the sample settle for 2-3 minutes.
- Filter at least 15 ml of the sample (Whatman 1) and collect the filtrate.
- Dilute the filtrate 1:10 with distilled water (ex. 100 µl of extract + 900 µl water).

7 WORKING SOLUTIONS PREPARATION

T2 Toxin Std: shake before use. Dilute standards 1:10 (1+9) with distilled water (ex. 50 µl standard + 450 µl water).
NB: standards should be diluted every analytical session in necessary amount.
Enzyme conjugate: ready to use.
Anti – T2 toxin Antibody: ready to use.
Developing solution: ready to use; this solution is light sensitive: keep away from direct light.
Stop solution: ready to use. Attention: contains hydrochloric acid 1N. Handle with care and in case of contact wash thoroughly with tap water.

8 ASSAY PROCEDURES

8.1 Preliminary comments

- Bring all reagents to room temperature before use and keep them at room temperature at least for an hour.
- Return al reagents to +2/+8°C immediately after use.
- Do not change the assay procedure, in particular:
  - do not prolong the first incubation time,
  - do not incubate the plate at a temperature higher than 25°C or lower than 18°C,
  - do not shake the plate during the incubations,
- Use for dispensing accurate and precise micropipettes with suitable tips.
- Once started, complete all the steps without interruption.
- The reproducibility of ELISA results depends largely upon the efficiency and uniformity of microwells washing; always keep to the described procedure;
- Use a single disposable tip for each standard and sample to avoid cross-contamination.
- Do not allow tips to contact the liquid already in the microwells.
- Avoid direct sunlight during all incubations. Is recommended to cover the microtiter plate without using sealing tapes.

8.2 Assay procedure

1. Predispose the assay layout, taking into account that standard and samples wells might be charged in double. Remove the wells and strips not to be used from the microtiter plate and replaced them in the pouch with the desiccant gel and reseal the pouch very well using the clump provided;
2. Add:
   - 50 µl of each standard/sample (previously diluted 1:10, see paragraphs 6 and 7) into the correspondent well;
   - 50 µl of enzyme conjugate in each well;
   - 50 µl of anti-T2 toxin antibody in each well using the multichannel micropipette and shake the plate gently with rotatory motion for a few seconds.
3. Incubate 10 minutes at room temperature.

Do not prolong the first incubation time and do not shake during incubation.

4. Washing sequence:
   - At the end of incubation, pour the liquid out from the wells;
   - Fill completely all the wells with distilled water using a squeeze bottle; pour the liquid out from the wells. Repeat the washing sequence for a total of 5 times;
   - Remove the remaining droplets by tapping the microplate upside down vigorously against absorbent paper.

Do not allow the wells to dry out.

5. Add 100 µl of development solution with the multichannel micropipette and mix thoroughly the plate with rotatory motion for a few seconds.
6. Incubate 5 minutes at room temperature protecting microplate from direct light.
7. Add 100 µl of stop solution to each well using multichannel micropipette and mix thoroughly the plate with rotatory motion for a few seconds.
8. Measure the absorbance at 450 nm. Read within 60 minutes.

9 RESULTS CALCULATION

- Divide the absorbance value of each standard and sample by the absorbance of the Standard 0 (B_0) and multiply by 100; the Maximum Binding (B_0) is thus made equal to 100% and the absorbance values are quoted as percentage:

$$\frac{\text{standard (or sample) absorbance}}{\text{Standard 0 } (B_0) \text{ absorbance}} \times 100 = \frac{B}{B_0} * 100$$

- Enter the B/B_0 values calculated for each standard in a semi-logarithmic system of coordinates against the T2 toxin standard concentrations and draw the standard curve.
- Take the B/B_0 value for each sample and interpolate it to the corresponding concentration in the calibration curve. Standards concentration already considers the sample dilution factor.

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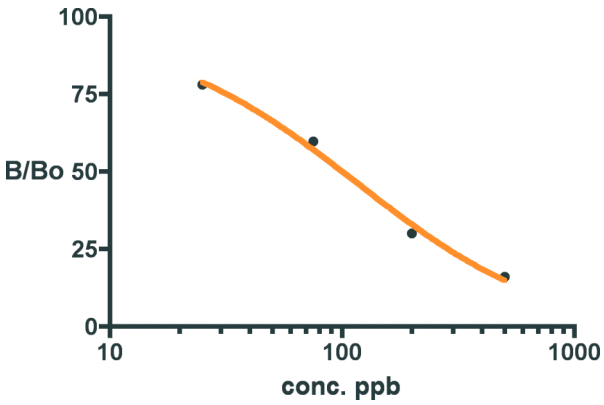
For the calibration curve it is suggested to use "4 parameters" curve or the "point to point" one. Excel spreadsheets can be downloaded from the section "download" in Tecna's web site, www.tecnalab.com

10 EXEMPLE OF STANDARD CURVE



11 RESULTS EVALUATION

After results elaboration, it is necessary to verify the assay performance. The verification is performed by comparison of obtained data with those given in kit specification (see paragraph 12). If the values are out from the specifications given, the assay result is doubt, and so the T2 Toxin concentration values in the sample could not be reliable. In this case it is advised to verify the expiry date of the kit, the wavelength of absorbance recording, as well as the procedure employed. If operation errors do not emerge, contact our technical assistance.
WARNING: substitution will be possible just in case of rendered kit. The kit must be conserved in its integral version and at the temperature indicated in this booklet.

12 KIT SPECIFICATIONS

Mean Bo absorbance	≥ 0,7 OD_{450nm}
B/Bo 50%	60 – 120 ppb
Standards duplicate C.V. mean	≤ 6%

13 LIABILITY

Samples evaluated as positive using the kit have to be re-tested with a conirmatory method.
Tecna shall not be liable for any damage to the customers caused by the improper use of the kit and for any action undertaken as a consequence of results.
Tecna shall not be liable for the unsafe use o the kit out of the current European safety regulations.

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