Celer T2

Enzyme immunoassay for the detection of T2 Toxin (Cat.nr. HU0040010 / HU0040030)

Celer T2 is a kit prepared for a competitive immunoenzymatic assay for the quantitative analysis of T2 and HT2 Toxins.
The kit contains the procedure and the materials sufficient for 96 determinations **(Cat.nr. HU0040010)** or 48 determinations **(Cat.nr. HU0040030)** including standards.
For the result evaluation a microtiter plate or strip photometer is required (manual or automatic ELISA reader).

Type of samples that can be analyzed (matrices)
Cereals (maize, wheat, durum wheat, oats, barley) and feed.

Sample preparation
Cereals and feed: grinding, extraction in methanol-water, filtration, dilution.

Assay time: 20 minutes (sample preparation not included).

Detection limit
Cereals and feed: 0.025 ppm

Specificity	
Analyte	**Cross-reactivity %**
T2 Toxin	100
HT2 Toxin	72
Deoxynivalenol (DON)	<1

1. TEST PRINCIPLE

The assay is performed in plastic microwells that have been coated with anti-T2 Toxin antibodies. In the premixing wells the enzyme conjugate and the standard solutions or samples are mixed and then transferred into the anti-T2 Toxin microtiter plate.
During the first incubation, free T2 Toxin in the standard solution /sample and enzyme-labelled T2 Toxin compete for the anti- T2 Toxin antibody binding sites on the solid phase. Any unbound enzyme conjugate and T2 Toxin molecule are then removed in a washing step.
The bound enzyme activity is determined adding a fixed amount of a chromogenic substrate. The enzyme converts the colourless chromogen into a blue product.
The addition of the stop reagent leads to a colour change from blue to yellow. The absorbance is measured with a microplate reader at 450 nm. The colour development is inversely proportional to the T2 Toxin concentration in the standard solution /sample.

2. PROVIDED REAGENTS

Premixing microtiter plate: non-coated wells, blank.
Microtiter plate: coated with anti-aflatoxin antibody.
As the strips are breakable, the wells can be used individually. For this purpose, it is sufficient to get out the wells from the frame and to break the joint.
T2 Toxin std: 5 plastic vials containing the standard solution in the following concentration: 0 ppm; 0.025ppm; 0.08 ppm; 0.4 ppm; 1 ppm of T2 Toxin.
Enzyme conjugate: 1 plastic bottle.
Washing-buffer 10x: 1 plastic bottle.
Developing solution: 1 plastic bottle.
Stop solution: 1 glass vial. White cap.

Component	Cat.nr. HU0040010 96 det.	Cat.nr. HU0040030 48 det.
Premixing microplate	96 wells (12 strips x 8 wells)	48 wells (6 strips x 8 wells)
Microtiter plate	96 wells (12 strips x 8 wells)	48 wells (6 strips x 8 wells))
T2 Toxin Std.	5 vials x 1,5 ml	5 vias x 1.5 ml
Enzyme conjugate	14 ml	8 ml
Washing buffer 10x	50 ml	50 ml
Developing solution	14 ml	8 ml
Stop solution	8 ml	6 ml

3. REQUIRED BUT NOT PROVIDED MATERIALS

- Distilled water.
- Mycotoxin Extraction Solution A" cat.nr. Eurofins Tecna ME070 or, as an alternative, methanol.
- NaCl

Equipment

- Balance
- For grinding: grinder or blender (like "Osterizer").
- For extraction (optional): shaker
- Filter paper (Whatman 1)
- 20-200 ul micropipette, tips
- 100-1000 ul micropipette, tips
- 50-300 ul multichannel micropipette, tips
- Microtiter plate, filter 450 nm.

4. WARNING AND PRECAUTIONS FOR THE USERS

- The test for *in vitro* diagnostic use only.
- Some reagents contain solutions that may be identified as dangerous substance by the Regulation (EC) No 1272/2008. Please refer to *Material Safety*

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Data Sheet available on both the Eurofins Technologies and Eurofins Tecna *(tecna.eurofins-technologies.com)* web site.
- Handle the reagents with caution, avoiding contact with skin, eyes and mucous membranes.

5. HANDLING AND STORAGE INSTRUCTIONS
- Store the kit at +2/+8 °C and never freeze.
- **Bring all reagents to room temperature before use (at least 1 hour). ATTENTION:** do not unseal the microplate until it reaches the room temperature.
- Reseal the unused strips of the microtiter plate in the bag together with the desiccant bag provided.
- Return all reagents to +2/+8 °C immediately after use.
- Do not use components after the expiration date.
- Do not intermix components from different kit lots.
- Do not use photocopies of the instruction booklet. Follow the original instruction booklet that is included with the kit.
- Do not change the assay procedure, in particular:
  - do not prolong the incubation times;
  - do not incubate the plate at temperatures higher than 25°C;
  - do not shake the plate during the incubations.
- Use accurate and precise micropipettes with suitable tips for dispensing.
- 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 solution and sample to avoid cross-contamination.
- Do not allow tips to contact the liquid already in the microwells.
- Avoid exposure to direct light during all incubations. It is recommended to cover the microtiter plate without using plate sealers.

6. SAMPLES PREPARATION
1) Mix carefully the sample to be analysed in order to make it homogeneous.
2) Finely grind the sample.
3) Weigh the sample, choosing among the options described in the following table:

Sample	NaCl	Extraction solution
50 g	10 g	250 ml 70% methanol
5 g	1 g	25 ml 70% methanol
50 g	/	250 ml 70% methanol, 4% NaCl*
5 g	/	25 ml 70% methanol, 4% NaCl*

*** Preparation of extraction solution with 70% methanol and 4% NaCl:**
For 100 ml of solution: dissolve 4 gr of NaCl in 20 ml of deionized or distilled water, add 70 ml of methanol, then add deionized or distilled water to 100 ml.

4) Shake thoroughly for 3 minutes.
5) Filter the sample and collect the filtrate. **ATTENTION:** filtration could be slow depending on the sample. It is suggested to let the sample settle a few minutes before filtration.
6) Dilute the extract 7 times (1+6) in deionized or distilled water, in order to obtain a dosage range 0.025-1 ppm.
7) If the sample is dosed >1 ppm, dilute the extract 5 times (1+4) in methanol 10%, in order to obtain a dosage range 0.125-5 ppm.

It is suggested to weigh 50 g in order to have a better representative analysis of the sample.

7. WORKING SOLUTIONS PREPARATION
T2 Toxin std: ready to use.
Enzyme conjugate: ready to use.
Washing buffer: dilute the concentrate 1:10 (1+9) with distilled water; **ATTENTION:** in presence of crystals, bring the solution at room temperature and stir in order to solve them completely.
The diluted washing buffer is stable at room temperature for 24 hours and at +2/+8°C for two weeks.
Developing solution: ready to use; this solution is light sensitive: keep away from direct light;
Stop solution: ready to use. **ATTENTION:** it contains 1 M sulphuric acid. Handle with care and in case of contact flush immediately with plenty of water.

8. ASSAY PROCEDURE
1) Predispose the assay layout, recording standard solutions and samples positions, taking into account that one well is required for each standard and sample. Prepare an equal number of premixing wells.
   ATTENTION: it is suggested to carry out no more than 48 determinations in each assay (standards included); if a multichannel pipette is not used, it is advised to carry out no more than 16 determinations in each assay (standards included).
2) First incubation
   - Add 100 µl of enzyme conjugate **in each premixing well**.
   - Add 50 µl of each standard/ sample into the corresponding premixing wells. The standard/sample contain high percentage of methanol: take care to rinse the tip pipetting up and down the solutions before adding to the wells.
   - Using the micropipette, mix the content of each premixing well (pipette up and down three times) and immediately transfer 100 ul into the corresponding anti-T2 Toxin antibody coated microwell.
   - **ATTENTION**: use new tips for each well to avoid cross-contamination.
   - Incubate 10 minutes at room temperature;
   - Do not prolong the first incubation time and do not use automatic shakers.
3) Washing

- Pour the liquid out from the wells.
- Fill completely all the wells with washing buffer 1x using a squeeze bottle. Pour the liquid out from the wells.
- Repeat the washing sequence three (3) times.
- Remove the remaining droplets by tapping the microplate upside down vigorously against absorbent paper.

Do not allow the wells to dry out.

4) Developing
   - Add 100 µl of developing solution to each well.
   - Mix thoroughly with rotatory motion for few seconds.
   - Incubate for 10 minutes at room temperature. Protect from direct light.
5) Add 50 µl of stop solution to each well and mix thoroughly with rotatory motion for few seconds.
6) Measure the absorbance at 450 nm.
7) Read within 60 minutes.

9. RESULTS CALCULATION

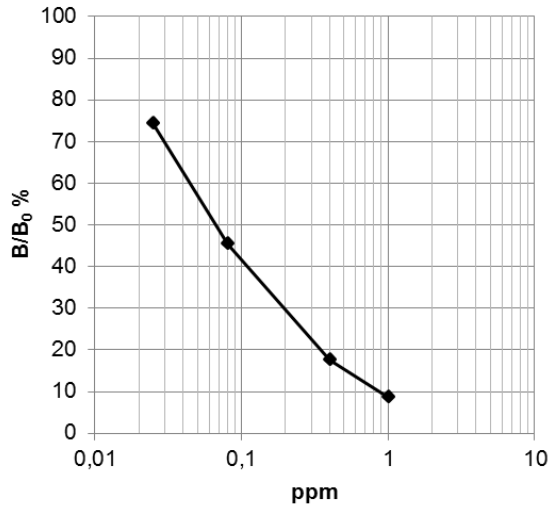
– Divide the absorbance value of each standard and sample by the absorbance of the standard 0 (B_0) and multiply by 100; the standard 0 (B_0) is thus made equal to 100% and all the other absorbance values are expressed as percentage:

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

- Enter the B/B_0 values calculated for each standard in a semi-logarithmic system of coordinates against the T2 Toxin standard concentration and draw the standard curve.
- Interpolate the B/B_0 value of each sample to the corresponding concentration from the calibration curve. Standards concentration (ppb) already considers the sample dilution factor.
- If the sample was 5 times further diluted in order to obtain a dosage range 0.125-5 ppm, multiply the result read on the calibration curve by a factor 5.

Please note: to build the calibration curve, the "spline" algorithm can be used, but it is also possible to use the "point to point" curve. To elaborate the ELISA results using the "point to point" method, Excel spreadsheet are available on Eurofins Tecna website tecna.eurofins-technologies.com and can be downloaded directly from the bottom of the product page.

10. CALIBRATION CURVE EXAMPLE



11. EVALUATION OF RESULTS

After processing the results, it is necessary to verify the assay performance. The verification is performed by comparison of obtained data with those given in kit specifications (see chapter 12).
If the values are outside the specifications given, then the results of the test are not assured, therefore the T2 Toxin concentration levels in the samples may not be valid.
In these cases it is advised to check the expiry date of the kit, the wavelength at which the reading was performed, as well as the procedure followed.
If operation errors are not identified as cause, contact our technical assistance.

WARNING: kit replacement will only be possible in case of return. The kit must be stored in its integral version at +2/+8°C.

12. KIT SPECIFICATIONS

12.1 Assay specification

B_0 absorbance	$\geq$ 0.7 OD_{450nm}
B/B_0 50%	0.04 – 0.15 ppm

12.2 Assay performance

Matrix	Cut off ppm	LOQ ppm
Maize	< 0.025	0.025
Whole grain wheat	< 0.025	0.025
White wheat flour	< 0.025	0.025
Oats	0.040	0.050
Durum wheat	< 0.025	0.025
Barley	< 0.025	0.050
Feed	0.050	0.050

	Recovery% (0.1-1 ppm T2 Toxin spiking)
Maize	109 ± 13
Whole grain wheat	125 ± 18
White wheat flour	116 ± 14
Oats	116 ± 10
Durum wheat	119 ± 17
Barley	117 ± 12
Feed	113 ± 21

The results were obtained by means of a "4 parameters" elaboration of the calibration curve.

13.LIABILITY

Samples evaluated as positive using the kit have to be re-tested with a confirmation method.
Eurofins Technologies Hungary shall not be liable for any damages to the customer caused by the improper use of the kit and for any action undertaken as a consequence of results.
Eurofins Technologies Hungary shall not be liable for the unsafe use of the kit out of the current European safety regulations.

14.LITERATURE

Rosar G., Persic L., Gon F., Puppini B., Bassani V., Diana. F. Analysis of mycotoxins in complex matrices by enzyme immunoassays. Poster presentation at 35th Mycotoxin Workshop, 2013, 22-24 May, Ghent, Belgium.

Gon F., Rosar G., Paoluzzi E., Diana F. Mycotoxin poly-contamination in maize: fast and sensitive ELISA test kits for a multi-analytical screening. Poster presentation at RME 2013, 21-23 January, Noordwijkerhout, the Netherlands.

Rosar G., Gon F., Puppini B., Baudino R., Rizzi N., Tosi G., Diana F. A new fast ELISA test kit for the quantitative detection of T2 and HT2 toxins: from the R&D development to the inter-laboratory trial. Poster presentation at WMF meets IUPAC, 5-9 Novembre 2012, Rotterdam, the Netherlands.

Rosar G., Gon F., Diana F. Sviluppo di un nuovo saggio ELISA per la determinazione della tossina T-2 avente elevata cross-reattività per la tossina HT-2. IV Congresso Nazionale: le micotossine nella filiera agro-alimentare e zootecnica. Istituto Superiore di Sanità. Roma, 11-13 giugno 2012. Rapporti ISTISAN 13/18, pag.144-150.