T-2 toxin (T2) ELISA Kit

Catalog Number. CSB-EFD027448

This immunoassay kit allows for the in vitro quantitative determination of T-2 toxin concentrations in feedstuff, feed, grain.

This package insert must be read in its entirety before using this product.

If You Have Problems

Technical Service Contact information

Phone: 86-27-87582341
Fax: 86-27-87196150
Email: tech@cusabio.com
Web: www.cusabio.com

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

INTRODUCTION

T-2 toxin is mycotoxin produced by several Fusarium species. Food crops as wheat, barely, corn, and their products are mainly contaminated. T-2 toxin is a threat for human health and animal husbandry. T-2 toxin primarily affects the blood, liver, kidneys, pancreas, muscles and function of the lymphocyte. The general symptoms of T-2 toxin poisoning are anorexia, vomiting, diarrhea, growth retardation, reproduction and neurological dysfunction.
This kit is a detection product developed based on ELISA technology, with operation time as short as 25 min and a sensitivity of 0.3 μg/kg, and linear range from 0.3 μg/kg to 8.1 μg/kg.

PRINCIPLE OF THE ASSAY

This assay employs the competitive inhibition enzyme immunoassay technique.
The microtiter plate provided in this kit has been pre-coated with T-2 toxin antigen. Standards or samples are added to the appropriate microtiter plate wells with T-2 toxin specific antibody and Horseradish Peroxidase (HRP) conjugated anti-antibody. The competitive inhibition reaction is launched between pre-coated T-2 toxin and T-2 toxin in standards or samples with the T-2 toxin special antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of T-2 toxin in the standards or samples. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

0.3 μg/kg-8.1 μg/kg

SENSITIVITY

The minimum detectable dose of the kit is typically less than 0.3 μg/kg.

The sensitivity of this assay, or Lower Limit of Detection (LOD) was defined as the lowest concentration that could be differentiated from zero. It was determined the mean OD value of 20 replicates of the zero standard added by their three standard deviations.

CROSS-REACTION RATE

T-2 toxin	100%

RECOVERY RATE

Feedstuff, feed, grain	70%-120%

LIMIT OF DETECTION

Feedstuff, feed, grain	10 μg/kg

PRECISION

Intra-assay Precision (Precision within an assay): CV%<10%
Three samples of known concentration were tested twenty times on one plate to assess.
Inter-assay Precision (Precision between assays): CV%<10%
Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Reagent	**Quantity(96T)**	**Quantity(48T)**
Assay plate	96T	48T
Standard	5 x 1 mL	5 x 0.5 mL
HRP-conjugate	1 x 7 mL	1 x 3.5 mL
Antibody	1 x 7 mL	1 x 3.5 mL
TMB Substrate	1 x 12 mL	1 x 6 mL
Stop Solution	1 x 10 mL	1 x 5 mL
Sample Diluent	2 x 50 mL	1 x 50 mL
Wash Buffer(10×)	1 x 30 mL	1 x 15 mL
Adhesive Strip	4	4
Instruction Manual	1	1

STANDARD CONCENTRATION

Standard	S1	S2	S3	S4	S5
Concentration (μg/kg)	0	0.3	0.9	2.7	8.1

STORAGE

Unopened kit	Store at 2--8°C. Do not use the kit beyond the expiration date
Opened kit	May be stored for up to 1 month at 2--8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- An incubator which can provide stable incubation conditions up to 25°C
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Centrifuge, Vortex mixer
- Analytical balance, 2 decimal place
- Absorbent paper for blotting the microtiter plate.
- Single-channel micropipette(20 µL-200 µL, 100 µL-1000 µL, 1000 µL-5000 µL)
- 300 µL multi-channel micropipette
- 100 mL and 500 mL graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.
- Ethanol.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Note:

- Kindly use graduated containers to prepare the reagent.
- Bring all reagents to room temperature (20-25°C) before use for 30 min.
- Only the disposable tips can be used for the experiments and the tips must be changed when used for different reagents.
- Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

REAGENT PREPARATION

- **Wash Buffer (1x)**: If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 10 mL of **Wash Buffer (10x)** into 90 mL deionized or distilled water to prepare 100 mL of **Wash Buffer (1x)**. Keep it at 4℃ for one month.
- **40% Ethanol**: Take 400 mL of **Ethanol** into 600 mL deionized or distilled water, shake well.

Note:

1. CUSABIO is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

SAMPLE COLLECTION AND STORAGE

Feedstuff, feed, grain

1. Weigh 5±0.05g of the homogenized sample, put into centrifugal tube.
2. Add 25 mL of **40% Ethanol**, vortex for 2 min.
3. Centrifuge at 4000 rpm for 5 min.
4. Transfer 50 µL of supernatant into a new centrifugal tube, add 950 µL of **Sample Diluent,** shake well.
5. Take 50 µL of sample for further analysis.
   Dilution factor of the samples: 100

STORAGE

The prepared sample may be stored for up to one day at 2-8°C.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature (20~25℃) before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 2-8°C.
3. Add 50 µL of **Standard** or **Sample** per well. Then add 50 µL of **HRP-conjugate** to each well and 50 µL of **Antibody** to each well. Cover the microtiter plate with a new adhesive strip and mix well, then incubate for 15 min at 25°C.
4. Aspirate each well and wash, repeating the process four times. Wash by filling each well with 250 µL of **Wash buffer(1x)** using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 30 seconds, complete removal of liquid at each step is essential to good performance.
5. Add 100 µL of **TMB Substrate** to each well, mix well. Incubate for 5 minutes at 25°C. Protect from light.
6. Add 50 µL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well within 5 min, using a microplate reader set to 450 nm (Recommend to read the OD value at the dual-wavelength: 450/630 nm within 5 min).

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 3 min. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding Substrates (e.g. observation once every 3 min). Substrates should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. Substrates are easily contaminated. Substrates should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the Substrates. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrates.

CALCULATION OF RESULTS

There are two methods to judge the results: the first one (A) is the rough judgment, while the second (B) is the quantitative determination. Note that the OD value of the sample has a negative correlation with T-2 toxin in the sample.

A:

Compare the sample average absorbance values with standards values, the T-2 toxin concentration in the samples can be concluded. For example, the absorbance value of sample 1 is 0.323, the absorbance value of sample 2 is 1.261; absorbance values of standard are:1.926,1.479,0.948,0.391,0.172 and the corresponding concentrations are:0 μg/kg, 0.3 μg/kg, 0.9 μg/kg, 2.7 μg/kg, 8.1 μg/kg; then the T-2 toxin in sample 1 and sample 2 are 2.7 μg/kg-8.1 μg/kg and 0.3 μg/kg-0.9 μg/kg; Lastly the reader is multiplied by the corresponding dilution factor of each sample and the actual concentration of sample is obtained

B:

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbency value}(\%) = \frac{B}{B_0} \times 100\%$$

B ——the average absorbance value of the sample or standard

B_0 ——the average absorbance value of the 0 μg/kg standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the T-2 toxin standards solution (μg/kg) as x-axis.

The T-2 toxin concentration of each sample (μg/kg), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each

sample followed, and the actual concentration of sample is obtained. (The software offered together will facilitate the calculation process, it's suitable for accurate and fast analysis of large scale samples, please contact us).

Note:

- Discard the substrate with any color that indicates the degeneration of this solution; when the absorbance value of S1 less than 0.5 indicates its degeneration.
- The optimum reaction temperature is 25℃, and too high or too low will result in the changes in the absorbance value and detecting sensitivity.

T-2 毒素(T2)酶联免疫试剂盒

使用说明书

【产品编号】CSB-EFD027448

【预期应用】ELISA 法定性（定量）测定饲料原料及成品、粮食、谷物等样本中 T-2 毒素的残留量。

【概述】

T-2 毒素（T2）是由多种镰刀菌产生的一种霉菌毒素。主要污染小麦、大麦、玉米等粮食作物及其制品，对人类健康及畜牧业构成了较大危害。T-2 毒素主要影响血液、肝脏、肾脏、胰腺肌肉及淋巴细胞的功能。T-2 毒素中毒后的一般临床症状为厌食、呕吐、腹泻、生长停滞、繁殖和神经机能障碍等。

本试剂盒是应用 ELISA 技术研发而成的检测产品，样本前处理简单，试剂盒操作时间仅需 25 分钟，灵敏度为 0.3μg/kg，线性范围为 0.3μg/kg～8.1μg/kg。

【实验原理】

本试剂盒采用间接竞争一步法。在酶标板中同时加入标准品（或样本）、酶标二抗及 T-2 毒素抗体，酶标板上包被的抗原与加入的标准品（或样本）中的抗原竞争结合加入的 T-2 毒素抗体，同时酶标二抗与 T-2 毒素抗体结合。用 TMB 底物显色，样本吸光值与其残留物中 T-2 毒素浓度呈负相关，与校准曲线比较再乘以其对应的稀释倍数，即可得出样本中 T-2 毒素的含量。

【产品性能指标】

1、 检测范围：0.3μg/kg – 8.1μg/kg

2、 灵敏度：0.3μg/kg

3、 精密度：批内差 CV%<10%，批间差 CV%<10%

4、 交叉反应率

T-2 毒素	100%

5、 回收率

饲料原料及成品、粮食、谷物等	70%-120%

6、样本最低检测限

饲料原料及成品、粮食、谷物等	10μg/kg

【试剂盒组成成分】

组份	96T	48T
酶标板 (Assay Plate)	96 孔	48 孔
标准品 (Standard)	5 x 1mL/瓶	5 x 0.5mL/瓶
酶结合物 (HRP-Conjugate)	1 x 7mL/瓶	1 x 3.5mL/瓶
抗体 (Antibody)	1 x 7mL/瓶	1 x 3.5mL/瓶
底物溶液 (TMB Substrate)	1 x 12mL/瓶	1 x 6mL/瓶
终止液 (Stop Solution)	1 x 10mL/瓶	1 x 5mL/瓶
样本稀释液（Sample Diluent）	2 x 50mL/瓶	1 x 50mL/瓶
浓缩洗涤液 (Wash Buffer)（10×）	1 x 30mL/瓶	1 x 15mL/瓶
板贴	4	4
说明书	1	1

【标准品浓度】

标准品	S1	S2	S3	S4	S5
浓度(μg/kg)	0	0.3	0.9	2.7	8.1

【存储条件及有效期】

1. 未开封的试剂盒避光保存于 2-8℃。有效期为 6 个月。请在试剂盒标注的有效日期内使用。
2. 酶标板打开后应置有干燥剂的铝箔袋中置于 2-8℃密封防潮保存，2-8℃条件下最多可保存一个月。

【所需仪器和试剂】

1. 酶标仪、离心机、恒温箱、涡旋仪、天平（感量 0.01g）、刻度移液管；
2. 微量移液器：单道 20μL-200μL、100μL-1000μL、1000μL-5000μL、多道 300μL；
3. 试剂：无水乙醇、去离子水。

【试剂配制】

1. **洗液工作液**：用去离子水将**浓缩洗涤液（10×）**按 1：9 体积比进行稀释。即 1 份**浓缩洗涤液（10×）**＋9 份去离子水。用于酶标板的洗涤，洗液工作液在 4℃环境下可保存一个月。
2. **40% 乙醇水**：取 400mL **无水乙醇**加入到 600mL 去离子水中，摇匀。

【注意事项】

1. 实验开始前，请提前配置好所有试剂，试剂或样品稀释时，均需混匀，混匀时尽量避免起泡。
2. 用户在初次使用试剂盒时，应将各种试剂管离心数分钟，以便试剂集中到管底。
3. 实验中必须使用一次性吸头，在吸取不同的试剂时要更换吸头。
4. 在配制检测溶液工作液时，请以相应的稀释液配制，不能混淆。
5. 工作液请依据所需的量配置使用。请勿重复使用已稀释过的工作液。

【样本前处理】

饲料原料及成品、粮食、谷物等样本的前处理

1. 称取 5±0.05g 磨碎的饲料样本于离心管中；
2. 加入 25mL **40%乙醇水**，振荡 2min；
3. 4000rpm 离心 5min；
4. 取 50μL 上清加入 950μL **样本稀释液**，混匀；
5. 取 50μL 稀释好的样本用于检测。

样本稀释倍数：100

【样品的保存】

处理好的样本可在 2~8℃保存 1 天。

【操作步骤】

1. 将各种试剂移至室温（20-25℃）平衡至少 30 分钟，按前述方法配制试剂，备用。
2. 按需要取出微孔条及板架，将不用的微孔条放回铝箔袋，保存于 2~8℃。
3. 每孔加**标准品**/混匀的**样品** 50μL，标准品/样品建议做复孔，然后加入**酶结合物** 50μL 和**抗体** 50μL，用板贴封板，轻轻震荡混匀，25℃反应 15 分钟。
4. 取出微孔板，甩干孔内液体，用稀释好的**洗液工作液**洗板 4 次。每次浸泡 30 秒，250μL/每孔，在吸水纸上拍干。
5. 显色：每孔加入**底物溶液** 100μL，轻轻震荡，25℃避光显色 5 分钟。
6. 依序每孔加**终止液** 50μL，终止反应（此时蓝色立转黄色）。终止液的加入顺序应尽量与底物液的加入顺序相同。为了保证实验结果的准确性，底物反应时间到后应尽快加入终止液。
7. 用酶标仪在 450nm 波长依序测量各孔的光密度（OD 值）。 在加终止液后 5 分钟以内进行检测（建议用双波长 450/630nm 检测，在 5 分钟内读完数据）。

【操作要点】

1. 为保证检测结果的准确性，建议标准品及样本均设双孔测定。每次检测均需做标准曲线。
2. **加样**：加样时，请使用一次性的洁净吸头，避免交叉污染。加样时应尽量轻缓，避免起泡，将样本加于酶标板孔底部，切勿沿孔壁加样。一次加样时间最好控制在 3 分钟内，如标本数量多，推荐使用排枪加样。
3. **温育**：为防止样本蒸发或污染，温育过程中酶标板必须覆上板贴，实验过程中酶标板应避免处于干燥的状态。温育过程中应随时观察温箱温度是否恒定，及时调整。温育过程中，温箱不易开启太多次，以免影响温度平衡。
4. **洗涤**：洗涤过程非常重要，不充分的洗涤易造成假阳性。Elisa 分析中的重现性很大程度上取决于洗板的一致性，请严格按照说明书要求洗板。
   a) 手工洗板方法：吸去（不可触及孔壁和孔底）或甩掉酶标板内的液体；在实验台上铺垫几层吸水纸，酶标板朝下用力拍几次；将洗液工作液按 250μL/孔注入孔内，浸泡 30 秒。根据操作步骤中所述，重复此过程 4 次。

b) 自动洗板：如果有自动洗板机，应在熟练使用后再用到正式实验过程中。

5. **显色**：为保证实验结果的准确性，底物反应时间到后应尽快加入终止液。可在加入底物溶液后每隔一段时间观察一下显色情况以控制反应时间（比如每隔 3 分钟）。当肉眼可见标准品前 3-4 孔有明显梯度蓝色，后 3-4 孔显色不明显时，即可加入终止液终止反应，此时蓝色立刻变为黄色。终止液的加入顺序应尽量与底物溶液的加入顺序相同。

6. 底物溶液应为浅蓝色或无色，如果颜色严重变深则必须弃用。底物溶液易受污染，请避光妥善保存。

7. 该试剂盒的最佳反应温度为 25℃，温度过高或者过低将导致检测吸光值和灵敏度发生变化。

【数据处理】

结果判定有两种方法，粗略判定可用第 1 种方法，定量判定用第 2 种方法。注意样本吸光值与其所含的 T-2 毒素含量呈负相关。

1、 粗略判定

用样品的平均吸光度值与校准值比较，可得出样本所含 T-2 毒素浓度范围（μg/kg）。假设样品 1 的吸光度值为 0.323，样品 2 的吸光度值为 1.261，标准品吸光度值分别是：0μg/kg 为 1.926、0.3μg/kg 为 1.479、0.9μg/kg 为 0.948、2.7μg/kg 为 0.391、8.1μg/kg 为 0.172，则样品 1 的浓度范围是 2.7μg/kg~8.1μg/kg；样品 2 的浓度范围是 0.3μg/kg~0.9μg/kg，再乘以其对应的稀释倍数即为样本中 T-2 毒素的实际浓度。

2、 定量分析

百分吸光度值的计算：所获得的每个浓度校准溶液和样本吸光值的平均值（B）除以第一个标准品（0 标准品）的吸光度平均值（B_0）再乘以 100%，即百分吸光度值。

$$\text{百分吸光度值（\%）} = \frac{B}{B_0} \times 100\%$$

B：标准品或样本的平均吸光度值

B_0：0μg/kg 标准品的平均吸光度值

标准曲线的绘制与计算：以标准品百分吸光率为纵坐标，以 T-2 毒素标准品浓度（μg/kg）的对数为横坐标，绘制标准曲线图。将样本的百分吸光率代入标准曲线中，从标准曲线上读出样本所对应的浓度，乘以其对应的稀释倍数即为样本中 T-2 毒素的实际浓度。若利用试剂盒专业分析软件进行计算，更便于大量样品的准确、快速分析，欢迎来电索取。

注：当标准品 **S1** 的吸光度值小于 0.5 时，表示该试剂盒可能变质。

【说明】

1. 本试剂盒仅供研究使用。
2. 中、英文说明书可能会有不一致之处，请以英文说明书为准。
3. 不同批号试剂不能混用。不要用其它生产厂家的试剂替换试剂盒中的试剂。
4. 刚开启的酶标板孔中可能会含有少许水样物质，此为正常现象，不会对实验结果造成任何影响。

Notes