COSMO BIO CO., LTD.
Inspiration for Life Science

Broad Check

『Deoxynivalenol (DON) ELISA』 Kit

Contents

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Research & Development
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I. Preface

Deoxynivalenol(DON) belongs to the trichothecene family mycotoxin and is most produced of *Fusarium graminearum*. DON often occurs in plant products particularly in cereal. Due to their high cytotoxic and immunosuppressive properties, the toxicological these toxins pose a risk to human and animal health. Accurate determination of the presence of toxin is of major importance to those monitoring the quality of feed and food in which DON may occur.We developed monoclonal antibody for DON , and a kit for detection of DON in cereal, feed with simple and easy procedure.

DON ELISA kit

▼ Measure range is 8.23－6000 ng/mL

▼ 40 samples can be measured with duplicate

▼ Microtiter plate is divisible strips, 96wells(12strips, 8wells each)

▼ Sampling volume is 50μL.

▼ Total reaction time is 1hr and 10 minutes.

A） Capture Ab coated plate
B) Standard
C) Enzyme conjugate solution
D) Enzyme conjugate diluent
E) Antibody solution
F) Substrate
G) Stop solution
H)Conc.washing solution
I) Plate cover

Storage and term of validity

The kit should be stored at 2 – 8℃

The kit is stable for 6 months after the date of manufacture.

Ⅱ Propeties

The kit is for quantitative detection of DON in cereal and feed and has outstanding specificity and quantification. The detection procedure is simple, easy and hardly influenced by other protein components.

Specificities

The ELISA kit is specific for DON and 15-acetyl DON (see Basic capability).

Assay principle

The ELISA kit adopts direct competitive reaction recognizing DON and their derivatives.

The capture antibody are coated on the surface of 96 well plate, onto which HRP-labeled DON, anti-DON antibody and DON standard solution or object to be measured are overlaid at once. After reaction, substrate is added to the wells, which reacts with the bound enzyme conjugate to produce blue color. The optical densities (OD) are measured and plotted the standard curve to calculate in the concentration of DON in the object to be measured.

Ⅲ Kit Contents

	Reagents	Standardized	Contents
A	Antibody coated plate	96 well plate 1plate	Capture antibody
B	DON standard	B0-B7 at each concentration per vial 8vials	DON
C	Enzyme conjugate solution	120u L 1vial	HRP-labeled DON
D	Enzyme conjugate solution diluent	6 mL 1vial	Phosphate buffered saline
E	Antibody solution	6 mL 1vial	Phosphate buffered saline containing Anti DON Antibody
F	Substrate for enzymes	12mL 1vial	3,3',5,5'-tetramethylbenzidine (TMB)
G	Stop solution	6mL 1vial	1N H_2SO_4
H	Concentrated washing solution	50mL 1vial	phosphate buffered saline containing Tween20
I	Plate cover	1	

Ⅳ. Preparation of sample

1) Wheat sample (*Contact Frontier Institute Co.,LTD http://www.frontier-science.co.jp/FIweb/top.html for silage extraction)

Weight 5g of ground sample and added it to suitable container(ex;

(50mL centrifuge tube) with 50mL of distilled water.

Shake vigorously for 3 minutes.

↓

Centrifuge (5000r.p.m, 5min.) and collect the supernatant

or

Filter by pouring through a Whatman No.1filter and collect the filtrate.

↓

The sample is ready for aasay.

1) * When the sample to be estimated is highly concentrated (>6000 ng/mL), the sample should be diluted with PBS.

V. Assay procedure

Materials required but not provided

1) Microtiter plate reader with 450nm filter.
2) Microtiter plate shaker (optional)
3) Microtiter plate washer or washing bottles
4) Micro pipette (20-100 μL) and tips
5) Micro dispenser (optional)
6) Scale marked cylinder (1000 mL)
7) Distilled water or deionized water

Preparation of reagent

1) Standard solution (B-0～B-7) are provided ready to use.

B 0 (0 ng/mL)
B 1 (8.23 ng/mL)
B 2 (24.7 ng/mL)
B 3 (74 ng/mL)
B 4 (222.22 ng/mL)
B 5 (666.66 ng/mL)
B 6 (2000 ng/mL)
B 7 (6000 ng/mL)

2) The enzyme conjugate solution (C, HRP-labeled DON 120 µL) should be diluted with the enzyme conjugate solution diluent for (D, HRP-DON diluent), and be used after sufficient stirring.
(After diluted, the solution is stable at 4℃ for 1week.)

If not use up the diluted solution within 1week, dilute only as needed.

ex; required 4 micro strips (32wells) for assay ;

HRP-labeled DON (C, 40 µL) should be diluted with
HRP-DON diluents (2mL).

3) Antibody solution (E, Anti-DON antibody) is provided ready to use.

4) The substrate for enzyme (F) should be used after enough equibration at the room temperature.
5) Stop solution (G) is provided ready to use.
6) The concentrated washing solution (50 mL) should be diluted with distilled water (450 mL), and be used to wash the plates.

Assay Protocol

1) All vials should be equibrated at the room temperature before use.
2) Pipet standard solution (50 µL) or the sample solution into each well, and add 50 µL of the HRP-labeled DON solution (C+D) into each well.
3) Add anti DON antibody (E, 50 µL) into each wells and mix by tapping the plate with finger (a plate shaker can also be used).
4) Cover the plate, and keep it at the room temperature (20～25℃) for 1 hour.
5) Dump the liquid out of the wells and fill the wells with the washing solution. Wash 3 times with the washing buffer solution, then turn the wells upside-down and tap out onto the paper towel until remaining washing solution has been removed.
When automatic washer is used, set 3 times wash with the washing solution (350 µL).
6) Add the substrate for enzyme (F, 100 µL) into each well, and keep the well at the room temperature (20～25℃) for 10 min to maintain a steady progress of reaction.
7) Add stop solution(G, 50 µL) to each wells.

8) Read the optical density (OD) of the wells using microtiter plate reader (450 nm filter). Standard calibration curve is obtained by the relation between the OD values and the standard concentrations of DON. Concentration of DON in the sample to be estimated can be read from the standard calibration curve.

Calculate the value for the standard by **4 paramete or log-logit formula.**

The DON concentration read from the standard curve must be multiplied by the dilution factor.

VI. Precautions

1) In the case of floating substance is observed in the prepared sample, additional centrifugation or sterile filtration is recommended before assay.
2) Reagents should be diluted immediately before use, and clean containers should be used in every step of preparation. Washing solution, however, can be kept at 4℃ for 6 months.
3) Precipitate may appear in the concentrated washing solution(H) sometimes during preservation, and can be dissolved during
   the dilution process.
4) New tip should be used for each sample to avoid from possible contamination.
5) When the sample to be estimated is highly concentrated(>6000ng/mL), the sample should be diluted with PBS.

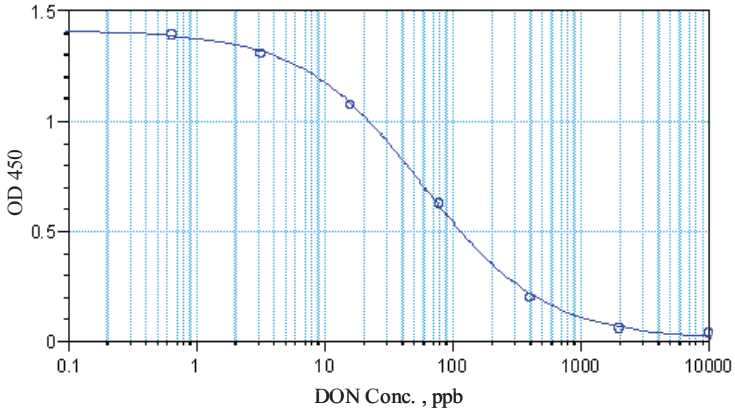
6) Recommend duplicate assay.
7) Read the OD immediately after stopping the reaction.
8) The standard curve should be made for each assay, since the lOD value can be slightly affected by reaction temperature, time cource, and degree of stirring.
9) Protect from strong light during assay and storage.

Consideration

a. Do not combine kits of different lot.
b. Research use only. Not for clinical diagnosis.

VII. Basic capabilities

Typical standard curve



Cross reactivity

DON	100%
15Ac-DON	120%
3Ac-DON	0.10%
NIV	5%
T2 Toxin	<0.1%

Reproducibility among simultaneous assay

CV(%)　3.2～6.8

Reproducibility among daily assay

CV(%)　4.8～8.6

Ⅶ.　Storage and term of validity

The kit should be stored at 2～8℃, and be stable within the expiration date.

Manufacturer

Frontier-Institute Co., Ltd

Address　1-777-12, SHINKO-NISHI, ISHIKARISI, HOKKAIDO,

Distributor

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