



**Enzyme Immunoassay for the quantitative analysis of
deoxynivalenol(vomitoxin)**

1、 General

Vomitoxin, named deoxynivalenol(DON) in chemistry, belongs to the trichothecene group of mycotoxins and is formed by fungi of the genus Fusarium. Deoxynivalenol often occurs in plant products particularly in cereals. Deoxynivalenol occurs in wheat, cereal grains, corn and malt often in the ppm range. Due to their high cytotoxic and immunosuppressive properties, not only a comprehensive routine check of the raw materials to be processed is required, but also of the final products.

The methods of detection used for deoxynivalenol are HPLC,GC and ELISA.

Using the ELISA, it is possible to detect deoxynivalenol in foods and feed rapidly and with accuracy.

2、 Test principle

The basic of the test is the antigen-antibody reaction. The microtiter wells are coated with DON-BSA conjugate. Monoclonal anti-DON and the DON standards or sample solution are added. Free DON and DON-BSA conjugate compete for the monoclonal anti-DON binding sites (competitive enzyme immunoassay).Any unbound monoclonal anti-DON is then removed in a washing step. Enzyme conjugate to sheep antibodies directed against rat IgG are added, and are bound by the immobilized monoclonal anti-DON. Any unbound enzyme conjugate is then removed in a washing step. Chromogen/substrate is added to the wells, bound enzyme conjugate converts the colorless chromogen into a blue product. The addition of the stop reagent leads to a color change blue to yellow. The depth of the color shows the magnitude of DON in the sample. The measurement is made photometrically at 450nm.According to the absorbance, the concentration of DON in the sample can be quantified.

The detected limit is 10 μ g/kg.

3、 Reagents provided

All reagents required for the enzyme immunoassay(including standards)are contained in the test kit.

We provide two specification of kits:24-well and 48-wells.

Each test kit contains:

1. microtiter plate; coated with antigen.24-well and 48-wells
 2. DON standard solutions
(0pp,12.5ppb,25ppb,50ppb,100ppb,200ppb).....red cap
 3. Monoclonal anti-DON(concentrate).....serum tube
 4. Sheep-anti-mouse-HRP conjugate(concentrate) serum tube
 5. Buffer solution.....brown cap
 6. Substrate solution.....blue cap
 7. Chromogen.....black cap
 8. Stop reagent.....yellow cap
-



9. Washing solution(concentrate)black cap
10. Microwell holder.....white

4、 preparation of samples

1. corn, feed and malt

--weight 5.0 g of ground mixed sample in a flask and fill up to 25 mL with 10% methanol.

--shake intensively for 10 minutes(manually or with shaker)

--filter the extract through filter paper.

--use 50 μ L of the filtrate per well in the ELISA test

2.beer and wort

--pipe some of beer sample

--shake intensively until the CO₂ is evicted out

--use 50 μ L of the filtrate per well in the ELISA test

Use the wort sample in the test directly.

Suggestion: asepsis-filter firstly if the sample is turbid.

5.Test procedure

5.1.Preliminary comments

1.Bring all reagents to room temperature before use.

2.According to the number of samples, extract corresponding amount of wells

3.The monoclonal anti-DON (monoclonal anti-DON, serum tube) is provided as a concentrate. For a satisfied result, only the amount that actually is needed should be reconstituted. The monoclonal anti-DON is dilluted 1:75 in buffer solution(For example, one tube of concentrate+1.5mL buffer solution, mixed thoroughly, sufficient for 24 wells).

4.The sheep-anti-mouse-HRP conjugate (sheep-anti-mouse-HRP conjugate, serum tube)is provide as a concentrate. For a satisfied result, only the amount that actually is needed should be reconstituted. The Sheep-anti-mouse-HRP conjugate should be shaken before pipetting. The Sheep-anti-mouse-HRP conjugate is dilluted 1:150 in buffer solution(For example, one tube of concentrate+1.5mL buffer solution, thoroughly, sufficient for 12 wells).

5.Dilute Washing solution concentrate with 300ml deionized water

5.2 Test procedure

1.Insert a sufficient number of wells into the microwell holder; record standard wells, and sample wells; wash each wells two times with washing solution, one minute each time, and then tap the microwell to ensure complete removal of liquid from the wells.

2.Add 50 μ l of DON standard solutions to comparison wells; 50 μ of sample solutions to sample wells.

3.Add 50 μ l of monoclonal anti-DON solution to the bottom of each well, mix thoroughly and incubate for 60 min at 37°C in the dark.

4.Pour the liquid out of the wells and tap the microwell holder vigorously to ensure complete removal of liquid from the wells. Washing the wells three times with washing solution,1 min each time, tap the microwell to ensure complete removal of



liquid from the wells.

5. Add 100 μ l of Sheep-anti-mouse-HRP conjugate solution to the bottom of each well, mix thoroughly and incubate for 30 min at 37°C in the dark.

6. Pour the liquid out of the wells and tap the microwell holder vigorously to ensure complete removal of liquid from the wells. Washing the wells five times with washing solution, 1 min each time, tap the microwell to ensure complete removal of liquid from the wells.

7. Add 50 μ l of chromogen and substrate solution, respectively, mix thoroughly and incubate for 10-15 min at 37°C in the dark.

8. Add 50 μ l of stopping reagent to each wells, mix well and measure the absorbance at 450 nm. Quantitative result can be confirmed.

6. Results

6.1. Semi-quantitative

Compare milk sample with standard solution (500ppb), while compare milk powder with standard solution (100ppb)

1. If the color of sample wells is deeper than standard solution (500ppb or 100ppb) wells, it indicates that:

the DON concentration in samples(ppb) \leq 500

2. If the color of sample wells is lighter than standard solution (500ppb or 100ppb) wells, it indicates that:

the DON concentration in samples(ppb) $>$ 500

3. If the color of sample wells is close to standard solution (0ppb) wells, it indicates that:

the DON in samples has not been detected

6.2. Quantitative

Made up DON standard solutions, the concentration of DON standard, the concentration of DON are 0, 10, 100, 500, 1000ppb, respectively, then the absorbance of each wells are measure photometrically at 450nm, draw the standard curve. The concentration of DON in samples can be quantified through the standard curve.

Standard curve: The absorbance values of standards (or samples) are divided by the absorbance value of the zero standard and multiplied by 100. The absorbance value of the zero standard and multiplied by 100. The absorbance value of the zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

Absorbance standard (or sample)

$\times 100 = \% \text{Absorbance}$

Absorbance zero standard

The values calculated for the standards are entered in a system of coordinates on semilogarithmic graph paper against the DON concentration (ppb).

The DON concentration in ng/ml corresponding to the extinction of each sample can be read directly from the standard curve.

Standard curve



Calibration curve of a DON kit

7.Warning and precautions

- 1.Store the kit at 2-8°C.Do not freeze.
- 2.Do not use DON kit past the expiration data. Dilution or adulteration of these reagents may result in loss of sensitivity.
- 3.The colorless chromogen is light sensitive therefore avoid exposure to direct light.
- 4.The standards contains DON, particular care should be taken. Avoid contact of the reagent with the skin.
- 5.Decontamination of the glassware and DON solutions is best carried out using a sodium hypochlorite solution (10%(v/v)) overnight (adjust solution with HCl to PH 7).

8.Refeences

- 1.Uslieber, E.et al. Direct enzyme-linked immunosorbent assays for the detection of the 8-ketotrichothecene mycotoxins deoxynivalenol,3-acetyldeoxynivalenol,and 15-acetyldeoxynivalenol in buffer solutions.[J] J.Agric.Food.Chem.1991,32:2091
- 2.Apsimon,J.W.et al. Mycotoxins from Fusarium species: detection determination and veritey.[J]Pure Apple. Chem.1990,62:1339