SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Deoxynivalenol (DON)ELISA Kit

Cat.No：SL0057Ot

1. Purpose

This kit is Deoxynivalenol (DON)Quantitative detection of residues in feed, fish, shrimp, tissue, egg, honey, milk, serum and urine sample.

2. Principle

This kit uses a direct competitive ELISA method in microplate coated with DON coupled antigen, adding DON standard or samples, free DON and pore strips Pre-coated DON conjugated antigen compete against DON antibody enzyme marker color with TMB substrate, stop solution added after the color from blue to yellow, with a microplate reader detection wavelength at 450nm, the sample absorbance value and DON content is inversely proportional to the sample through the standard curve DON content.

3. Kit composed

(1) Pre-coated DON coupled antigen removable microtiter plates: 1 block (12wells×8strips)
(2) DON Standard: 6bottles (1ml/vial), contents were: 0ppb, 1ppb, 3ppb, 9ppb, 27ppb, 81ppb.
(3) Anti-DON antibody conjugate: 1 bottle (6ml)
(4) Color liquid A: 1 bottle (6ml)
(5) Color liquid B: 1 bottle (6ml)
(6) Stop Solution: 1 bottle (6ml), 2M sulfuric acid.
(7) Sample Diluent: 1 bottle (10×, 6ml), used for sample dilution.
(8) washing liquid concentrate: 1 bottle (20×, 20ml), for the washer
(9) A description

4. The material needs without providing

(1) Equipment

1) Wavelength 450nm microplate reader.
2) Mill.
3) Graduated cylinder.
4) Oscillator.
5) Funnel.
6) Whatman No 1 filter paper or equivalent.
7) Pipette.

(2) Reagent

1) Deionized water or distilled water.
2) Methanol.

5. Notes

—Using the kit, please carefully read the instructions before
—Do not use expired kits.
—Kit Before use, the reagents to room temperature (25±2°C), recommend at least 2 hours back to temperature.
—Standard contains DON, use with caution, the operation should wear gloves.
—Stop solution contains sulfuric acid, used clothing to prevent skin burns and corrosion.
—Different standards, the sample used in the nozzle can not be mixed, otherwise it will affect the test results.
—Different lots of reagent kit not mix; different standard samples are not mixed with the tip, otherwise it will affect the experimental results.
—Diluted samples must be used in the sample kit diluent, otherwise it will affect the experimental results
—Avoid mixing foaming agent. Kit stored at 2~8°C, not frozen, not dried out of the microplate should be kept sealed

6. Working fluid

(1) DONstandard solution: 0 ppb, 1ppb, 3ppb, 9ppb, 27ppb, 81ppb.
(2) Concentrated washing solution: distilled water ratio of 1:20 (1+19) dilution reserve
(3) Sample Diluent: distilled water ratio of 1:10 (1+9) dilution reserve
(4) Color reaction: is standby to prevent direct rays of light
(5) Reaction termination solution: have spare

7. Sample handling procedures

(Sample during the extraction process, we must strictly follow operating instructions should be accurately dilute the extraction process, it will lead to inaccurate results, samples should be stored in a cool dark place and kept refrigerated)
(1) 10g crushed samples taken, add 20ml 70% methanol solution
(2) Strong 3-minute oscillations
(3) With Whatman No 1 filter paper
(4) take 100 apms l treatment of samples, join 400 apms l samples diluent
(5) take 50μl sample dilutions analysis (sample diluted times for 5) .

8. Elisa analysis steps

(1) Experimental Notes

1) Keep all reagents before the start of the experiment outside the box to fully restore to room temperature (25±2°C), about 2 hours. Warmed to room temperature (25±2°C) before removing the porous section, the excess pore re-sealed immediately in Article 2~8°C drying saving

Note: You must ensure the return to full temperature, or the accuracy of detection and accuracy.

2) immediately after use reagents stored back into the 2~8°C

3) Do not change the analysis program

4) Use the exact pipette

5) Operation once started, please do not interrupt any program

6) ELISA results of the reproducibility of a great extent depends on the operating program, strictly in accordance with the requirements of operation

7) To avoid cross-contamination, each standard and sample should be added using a different kind of tip

8) Do not add to the tip sample contact with the porous surface of the solution

(2) Analysis of the steps

1) Pre-numbered, labeled B0, standard and sample location, detecting holes is recommended

2) Take the required amount of micropore (pore removable section), the excess back into the strip re-sealed and immediately save 2~8°C

3) Sample dilution (10×), concentrated washing liquid (20×) was diluted into the working fluid

(distilled or deionized water to dilute)

4) Hole in the B0 adding 50µl 0 ppb standard solution

5) Add 50µl of the standard holes in the standard solution

6) Hole in each sample by adding 50µl sample solution

7) In all holes by adding 50µl of Anti- DON antibody conjugate

8) Gently shake the plate for several seconds

(3) 37°C temperature bath 30min

(Warm bath tap from time to time during the reaction plate, holes can reduce the error)

1) Get rid of holes in the liquid, wash with a lotion microplate 5 times, the last one should beat the absorbent paper to remove the holes in the liquid completely

(4) Reaction

1) The washing process is complete, immediately pipette in each pore of color to join the 50µl solution A, add 50µl color liquid B; mild shaking plate to mix thoroughly

2) 37°C 10min warm bath

3) Each well add 50µl stop solution, mix

4) The absorbance at 450nm under the test results be read in 5min.

9. Calculation of results

(1) Quantitative Analysis

1) Obtained samples of each concentration of standard solution and the mean absorbance value (B) divided by a standard (0 standard) of the absorbance value (B0) and multiplied by 100%, that percentage of the absorbance values. B-standard solution or sample solution, the average absorbance B0-0 ppb standard solution of the mean absorbance values

2) DON concentration on the value of X axis, Y axis is percentage of absorbance, the standard curve graph. Percentile according to the sample absorbance value can be obtained from the corresponding point on the curve the abscissa, that is, DON concentration on the values obtained shall be measured against the number of medium DON concentration C (ppb)

3) After a pre-dilution of the sample, so the standard curve derived by multiplying the sample concentration dilution factor will again.

(2) Semi-quantitative determination

1) The visual semi-quantitative determination: first select an appropriate standard solution and sample with the operation, according to the sample absorbance with the standard level of comparison, to determine the sample concentration is less than or greater than the standard value.

2) Semi-quantitative measurement instrument: first select an appropriate standard solution and sample with the operation, according to the color depth of the sample compared with the standard
to determine sample concentration value is less than or greater than the standard value.

10 .specific

Material cross-reactivity.

DON……100%

11. Kit Parameters

—This kit detection limit 1ppb.

—B0 best value should be greater than 1.0 absorbance

—Absorbance plate kit error of less than 8%, less than 15% between the plates.

—With the manual extraction of tissue samples provided by more than 80% recovery rate.

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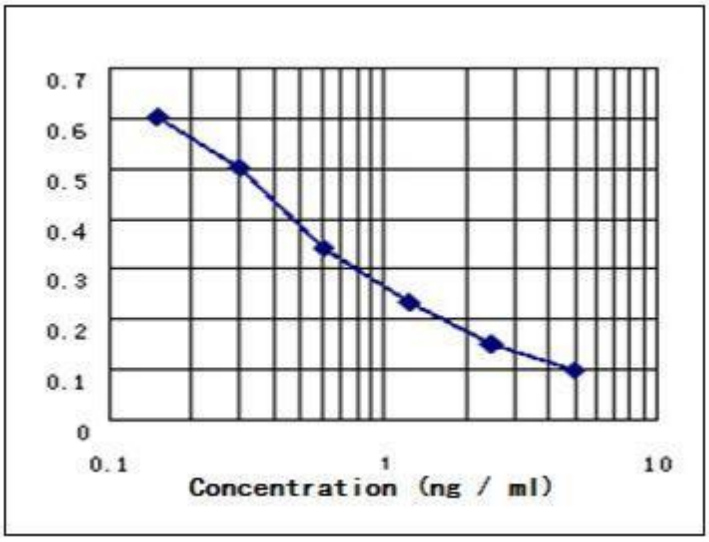
12. Standard curve mode

Kit provided by the standard curve range 1ppb~81ppb.



13 Analysis of restriction

This kit samples test positive should be another way, such as HPLC or GC / MS to be