

1. Composition of the reagent kit:

- 1) microtiter plate; coated with antigen.24-well and 48-wells
- 2) zearalenone standard solutions(0,1,2.5,5,10,20 μ g / L) 6 Bottles
- 3) sample diluent..... 1 Bottles
- 4)the first antibody concentrated solution: anti-zearalenone antibody.....1 / 2 bottle
- 5) the first antibody dilution..... 1 bottle
- 6) labeled the anti-concentrated liquid..... 1 / 2 bottle
- 7) The second antibody dilution..... 1 bottles
- 8) chromogenic substrate of a..... 1 bottle
- 9) chromogenic substrate solution b..... 1 bottle
- 10) Termination of..... 1 bottles
- 11) concentrated detergent solution (20 \times)..... 1 bottles
- 12)reaction plate stent..... 1 block

2. Apparatus and equipment configuration

- 4.1 micro pipettes, range 20 to 200 μ l (if possible, matching eight or 12 micro pipettes)
- 4.2 Meibiaoyi (with 450 nm wavelength)
- 4.3 or heterogeneity of the small mill
- 4.4 Centrifuge
- 4.6 Fridge
- 4.7 Analytical Balance
- 4.8 glassware: Erlenmeyer flask, beakers, crushed the mantle of the dropper of liquid such as shift

3. Operational flowchart

Sample 37 $^{\circ}$ C, 60 minutes 37 $^{\circ}$ C, 30 minutes 37 $^{\circ}$ C, 15 minutes

→ sample extraction reagent preparation → immune response - → enzyme reaction - → two-color reaction ----- → results found

4. Sample processing

Cereals and feed samples

Said admitted smashing sieved (20 mesh) in the sample with 5.0 g Cypriot Sanjiaoping, by adding 25% methanol mL50 - aqueous solution of Cyprus fully Zhenyao five minutes, resting for a while, filtration. Admission of a certain volume of liquid sample dilution with appropriate dilution (for example, 10 times), which is kind of analyte. Kind of analyte from 50 μ L directly in the porous sample, conducted ELISA testing.

5. Experimental preparations

7.1 concentrated liquid detergent diluted 20 times with distilled water, diluted before use. (For example: 3 mL concentrated washing liquid +57 mL of distilled water, adequate use of hole 24)

7.2 Preparation of the first antibody: before use, the first antibody from a bottle of concentrated liquid and accurately by adding 1.5 mL first antibody dilution, blending, and the preparation of the first experimental antibody solution, enough 24-hole plate reaction use.

7.3-2 enzyme preparation methods: the use of the former, two from a bottle labeled anti-concentrated liquid and accurately by adding 5.0 mL second antibody dilution, blending, and the preparation of two experimental anti-labeled solution, enough 24-hole plate reaction use.

6. Experimental steps

8.1 Reagent balance: will test box removed, placed more than 15 minutes, the balance to room temperature.

8.2 holes #: shift from the required response porous plate placed on the stent, not washing. Set on the 1st hole for Meibiaoyi zero on the 7th hole, 2-for zearalenone-control standards, and the remaining samples for the hole.

8.3 immune response: series of steps shown by the order by adding good solution and sample preparation solution.

Step 1: on the 1st hole by adding 50 μ L sample dilution ,2-on the 7th hole by adding 50 μ L Series concentration (0,0.1,0.5,1,5,10 μ g / L) standard, and the remaining holes by adding the corresponding sample extract.

Step 2: on the 1st hole by adding 50 μ L antibody dilution, in all other microporous adding 50 μ L first antibody solution.

Step 3: gently Zhenyao, the hole in the reaction of blending.

Step 4: 37 $^{\circ}$ C, and incubated 60 minutes.

Table of reaction

Operation of the order amount hole

1 2 3 4 5 6 7 8

First 50 μ L sample dilution 0 0.1 0.5 1 5 10 samples

The second step, 50 μ L antibody dilution of the first antibody

The third step Yaoyun

Step 4 37 $^{\circ}$ C, and incubated 60 minutes

8.4 Washing: Remove plate liquids, each hole by adding 250 μ L washing liquid, lotion not overflow, placed one minute, get rid lotion, absorbent paper in the shoot dry, repetitive washing three times.

8.5-2 enzyme reaction: each hole by adding 100 μ L-2 enzyme solution, 37 $^{\circ}$ C, incubated 30 minutes.

8.6 Washing: Remove plate liquids, each hole by adding 250 μ L washing liquid, lotion not overflow, placed one minute, get rid lotion, absorbent paper in the shoot dry, repetitive washing five times.

8.7 in each of the last 50 holes to add a μ L substrate, and then joined 50 μ L b substrate solution, blending in 37 $^{\circ}$ C incubator in color for 15 minutes.

Microporous 8.8 in each adding 50 μ L termination of reaction.

8.9 microplate gently shaken, 30 minutes of the reader at 450 nm optical density (OD).

7. Results found

Experimental determination of the above steps of the 3-7 hole OD value on the 2nd hole and the OD value multiplied by the ratio of 100 as a longitudinal coordinates to the concentration of the standard solution (lgC) as abscissa, a standard curve can be drawn Fig.

OD value of samples hole on the 2nd hole and the ratio multiplied by 100, check standard curve, the corresponding samples can be used on the numerical concentration lgC, seeking their opposition number of samples can be obtained in the extract of zearalenone content, in order to obtain samples of concentration, the concentration of readings must be multiplied by the corresponding dilution factor.

$$X = C \times V \times n$$

M

X - samples zearalenone content (ppt)

C - from the standard curve on the investigation should be relatively sample concentration (ppt)

V - extract sample volume (25 mL)

M - the quality of samples (5 g)

N - sample extract dilution