# Zearalenone (ZON/ZEN) Rapid Test Kit

Technical Manual

(GICA)

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## 1 Principle and Application

The test kit is used for detecting Zearalenone(ZEN) in the sample of grain, feed and others.

The kit is developed using colloidal gold immunochromatography assay (GICA) based on competition. After the sample solution is added to sample hole, if ZEN is present, it will bind with gold labeled antibodies, thereby preventing the labeled antibodies from binding to the ZEN conjugates on the nitrocellulose membrane. The results are judged according to the contrast of color strength.

## 2 Technique Data

**Detection Limits:** 60-1500ppb(ppb=μg/Kg)

## 3 Kit Content

| Package specification | 20T/Kit | 40T/Kit |
|---|---|---|
| Test device (with disposable dropper) | 20 | 40 |
| ZEN Reconstitution Buffer | 30mL×1 | 30mL×2 |
| Instruction | 1 | 1 |

## 4 Materials Required but Not Supplied

**4.1 Equipment:** grinder (for crushing solid samples), vortex mixer (for shake and mix), centrifuge, graduated transfer pipette, and balance with a division value of 0.01 g.

**4.2 Micropipettes:** single-channel (5-50μL, 20-200μL and 100-1000μL).

**4.3 Reagents:** Ethanol.

## 5 Sample Pre-treatment

Please note that the labware must be clean. Use disposable droppers to avoid contamination of interference results.

**5.1 Solution preparation before sample pre-treatment**

**Solution 1:** Sample extraction solution:

50% Ethanol solution, (Ethanol /Deionized water= 1: 1).

**5.2 Sample pretreatment step:**

**5.2.1 Sample treatment**

Weigh 5.0±0.05g of crushed samples into a 50mL centrifuge tube, add corresponding extraction fluid according to the table below, shake for 5 min and centrifuge at 4000 r/min for 5 min at room temperature or stand until layered. What we get is the supernatant.

| Sample | Extraction fluid | Amount of extraction fluid (mL) |
|---|---|---|
| General grain, feed | 50% Ethanol solution( Solution 1) | 12 |
| Samples with strong water absorption | 50% Ethanol solution( Solution 1) | 24 |
| Soybean, soybean meal | Ethanol | 12 |

**5.2.2 Dilute the sample**

**1. Detection limits:** 60-500ppb(ppb=μg/Kg)

**Sample 1: General grain, soybean and other feed raw materials**

Take 1mL of ZEN Reconstitution Buffer, add the supernatant (Obtained from 1. Sample treatment) according to the following method, mix them well, and wait for testing.

| Detection Limits | 60ppb | 100ppb | 250ppb | 500ppb |
|---|---|---|---|---|
| supernatant | 90μL | 55μL | 20μL | 10μL |

**Note:** The supernatant of highly absorbent samples needs to be doubled in addition.

**Sample 2: Corn, corn by-products and other feed products**

Take 1mL of ZEN Reconstitution Buffer, add the supernatant (Obtained from 1. Sample treatment) according to the following method, mix them well, and wait for testing.

**Note:** The supernatant of highly absorbent samples and corn gluten meal needs to be doubled in addition.

| Detection Limits | 100ppb | 250ppb | 500ppb |
|---|---|---|---|
| supernatant | 35μL | 15μL | 10μL |

**2. Detection limits:** 1000-1500ppb(ppb=μg/Kg)

Take 1mL of ZEN Reconstitution Buffer, add 10μL of the supernatant (Obtained from 1. Sample treatment) (For supernatant of highly absorbent samples and corn

gluten meal, take 20μL of the supernatant) and shake well to obtain a mixed solution. Take another 200 μL of ZEN Reconstitution Buffer for secondary dilution according to different detection limit requirements in the following table, mix them well, and wait for testing.

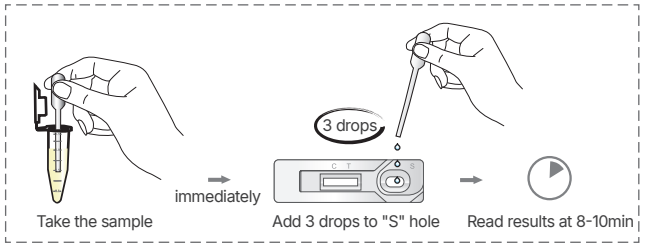
| Detection Limits | 1000ppb | 1500ppb |
|---|---|---|
| Mixed solution | 200μL | 100μL |

## 6 Test Steps

1)Tear the foil pouch, take out of the test card, and put on a flat, clean work surface.

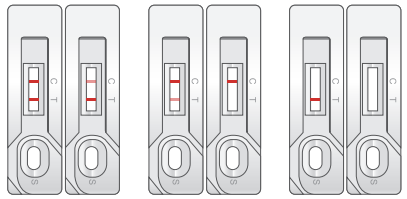
2)Pipette the prepared sample solution with the provided dropper, then add 2-3 drops (approximately 60μL) vertically and slowly into the sample hole(“S”).

3)Read the result at room temperature in 8 to 10 minutes. Results over 10 minutes can only be used as reference.




## 7 Results Judgement

**Negative:** Test ( “T” ) line and control( “C” ) line both appear in the result window. The color of the test ( “T” ) line is consistent or deeper than the control( “C” ) line. It indicates that the concentration of ZEN in the sample is below the detection limit, or absent.




**Positive:** In the result window, the control( “C” ) line appears, while the Test( “T” ) line does not appear or appears lighter in color than the control( “C” ) line. It indicates that the concentration of ZEN in the sample is above the detection limit.

**Invalid:** If the control( “C” ) line does not appear, the result might be considered invalid.

## 8 Notice

8.1 Don’t use the expired or damaged products.

8.2 When the test card is taken out of the refrigerator, it should be restored to the room temperature and then opened. The opened test card should be used as soon as possible to avoid failure after being affected by moisture.

8.3 Avoid touching the white nitrocellulose membrane in the middle of the detection card.

8.4 In order to avoid cross-contamination, the droppers cannot pipet another Solution after pipetting one.

8.5 The sample solution to be examined needs to be clear and free of turbid particles. Otherwise, it is prone to lead to blockage, non-obvious color development and other abnormalities, affecting the determination of the experimental results.

## 9 Storage Conditions

The kit shall be stored at 2°C to 30°C (35.6°F to 86°F) in dry environment.

Shelf life: 12 months. The date of manufacture is presented in the label of the box.