Elabscience®

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# NMZ(Nitiomidazoles) ELISA Kit

Catalog No: E-FS-E035

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

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Please kindly provide us the lot number(on the outside of the box) of the kit for more efficient service.

## Test principle

This kit uses Indirect-Competitive-ELISA as the method. It can detect NMZin samples, such as honey, tissue, etc. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with NMZ. During the reaction, NMZ in the samples or standard competes with NMZ on the solid phase supporter for sites of NMZ antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of NMZ. You can calculate the concentration of NMZ in the samples by comparing the OD of the samples to the standard curve.

## Technical indicator

**Sensitivity:**0.5ppb(ng/ml)

**Reaction mode:** 25℃ 30min～30min～15min

**Detection limit:** Tissue(Chicken, duck meat/Liver, Fish, shrimp, etc.)--- 0.25ppb
Honey---0.25ppb

**Cross-reactivity:** Metronidazole (MNZ) ---100%,
Dimetridazole (DMZ) ---68%,
Ronidazole(RNZ) ---112%
Ipronidazole ---33%

**Sample recovery rate:** Fish/Shrimp/ poultry/Live……90%±10%
Honey samples ……90%±10%

## Kits components

| Item | Specifications |
|---|---|
| Micro ELISA Plate | 96 wells |
| Standard Liquid(6 bottles, black cap) | 1mL each<br>0 ppb、0.5ppb、1.5ppb、4.5ppb、13.5ppb、40.5ppb |
| High Standard (100ppb) | 1mL |
| Antibody Working Solution(blue cap) | 5.5mL |
| HRP Conjugate(red cap) | 11mL |
| Substrate Reagent A(white cap) | 6mL |
| Substrate Reagent B(black cap) | 6mL |
| Stop Solution(yellow cap ) | 6mL |
| 20×Concentrated Wash Buffer(white cap) | 40mL |
| 2×Concentrated Re-dissolveSolution(yellow cap ) | 50mL |
| Product Description | 1 copy |

## Other supplies required

**Instruments:**Microplate reader, Printer, Homogenizer, Nitrogen Evaporators, Oscillators, Centrifuge, Graduated pipette, Balance(sensibility 0.01g).

**High-precision transferpettor:** single channel(20-200μL, 100-1000μL), Multichannel (300μL).

**Reagents:** NaOH,Anhydrous sodium carbonate, Sodium bicarbonate, N-hexane, Ethyl acetate.

## Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate reader in advance, preheat the instrument, and set the testing parameters.

**1. Sample pretreatment**

**Notice:** experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

**2. Solution preparation**

Solution 1: 0.1M carbonate buffer solution

Dissolve 4.66g Anhydrous sodium carbonate and 0.5g Sodium bicarbonate with 500mL deionized water. pH= 10.6

Solution 2: 2M sodium hydroxide solution

Dissolve 40g sodium hydroxide with 500ml deionized water

Solution 3: Re-dissolve solution

Double dilute the 2×Redissolve buffer with deionized water.

(2×Redissolved buffer (V): deionized water (V) = 1:1, Redissolved buffer can be stored for 1 month at 4℃ temperature.

Solution 4: Washing BufferDilute 20×Concentrated Wash Buffer with deionized water

20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19.

**3. Sample pretreatment procedure**

**Pretreatment ofTissue (Chicken, duck meat/Liver, Fish, shrimp, etc.), Honey**

(1) Weigh 3g homogenatesamples, add 3mL 0.1M carbonate buffer solution, oscillate and dissolvethoroughly.

(2) Add 9mlEthyl acetate, oscillate for 5min.Centrifuge at 4000r/min at room temperature for 5min.

(3) Take 6mL of upper liquid to another tube, add 2ml Ethyl acetate and 2ml 2M sodium hydroxide solution, oscillate for 5min, centrifuge at 4000r/min at room temperature for 5min.

(4) Take clean 4ml of upper liquid to another clean glass tube, blow-dry in 30-40℃nitrogen or air

(5) Add 1ml N-hexane, vortex for 30s, add 0.5ml redissolved buffer, mix for 30s. Centrifuge at 4000r/min at room temperature for 5min.

(6) Discard the upperorganic phase, take 50uLlower liquid foranalyze.

**Note: dilution ratio: 0.5, minimum detection dose: 0.25ppb**

## Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature before use.**All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**Dilute 40mL of concentrated wash buffer into 800mLwash working buffer with deionized or distilled water.

1. **Number:** number the sample and standard in order(multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50μL of Standard, or Sample per well, add 50μL of antibody working solution, cover the plate with sealer we provided, oscillate for 5s gently to mix thoroughly, incubate for 30 min at 25℃in the dark.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 250μL of washing buffer to each well and wash. Repeat wash procedure for 5 times, 30s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Add HRP conjugate:** Add 100 μL HRP conjugate to each well, incubate for 30min at 25℃in the dark.
5. **Wash:** Repeat step 3.
6. **Color Development:** add 50μL of substrate solution A to each well, and then add 50μL of substrate solution B. Gently oscillate for 5s to mix thoroughly. Incubate shading light for 15min at 25℃(The reaction time can be extended according to the actual color change).
7. **Stop reaction:** add 50μL of stop solution to each well, oscillate gently to mix thoroughly.
8. **OD Measurement:** determine the optical density(OD value) of each well at 450nm with a micro-plate reader (the 450/630 nm double wavelength is recommended).This step should be finished in 10min after stop reaction.

## Result analysis

1. **Absorbance (%)=$A/A_0$×100%**

   A: Average absorbance of standard or sample

   $A_0$: Average absorbance of 0 ppb Standard

2. **Drawing and calculation of standard curve**

   Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance value of sampleto standard curve to get corresponding concentration.If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

   For this kit, it is more convenient to use professional analysis software for accurate and fast analysis on a large number of samples.

## Notes

1. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperature is below25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor repeatability, move on to the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. Micro ELISA plate should be covered by plate sealer.Avoid the reagents to strong light.
5. Do not use expired kit and reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)is below0.5 unit($A_{450nm}$＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

## Storage and valid period

**Storage**: Store at 2-8℃. Avoid freeze / thaw cycles.

**Valid Period**: 1 year, production date is on the packing box.