

# Nitroimidazoles (NMZ) ELISA Kit

Technical Manual  
(ELISA)



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## 1 Principle and Application

This kit adopts the method of indirect competitive enzyme-linked immunoassay (ELISA) to detect Nitroimidazoles (NMZ) in the sample such as tissue, honey, milk and eggs. The kit is composed of Microtiter Plate coated with coupled antigens, HRP enzyme conjugate, antibodies, standards and other supporting reagents. During the detection, with adding standards or samples, the NMZ in the samples will compete with the coupled antigens to combine with anti-NMZ antibodies. After adding HRP conjugate, take coloration with TMB substrates. Absorbance value of the samples is a negative correlation with NMZ content. Lastly, by comparing the obtained absorbance values with the standard curve, we can calculate the NMZ content in the sample.

## 2 Technique Data

**2.1 Kit Sensitivity:** 1.5ppb (ng/mL)

**2.2 Reactive Mode:** 25°C, 30min ~ 30min ~ 15min

**2.3 Detection Limits:**

Sample	Detection Limits
Tissue (chicken, duck, liver, fish, shrimp, etc.)	1.5ppb
Honey	1.5ppb
Milk	1.5ppb
Eggs	3ppb

**2.4 Cross-reaction Rate:**

Drug name	Cross-reaction Rate
Metronidazole (MNZ)	100%
Dimetridazole(DMZ)	68%
Ronidazole(RNZ)	112%
Ipronidazole(IPZ)	33%

**2.5 Sample Recovery Rate:**

Sample	Recovery rate
Tissue (Fish, shrimp, poultry, liver, etc.)	90±10%
Honey, milk, eggs	90±10%

## 3 Composition of the Kit

Reagent	Specification
Microtiter Plate	8wells× 12strips
Standard: 0ppb, 1.5ppb, 3.0ppb, 6.0ppb, 12.0ppb, 24.0ppb (black cap)	1.0mL each
High Standard: 100ppb (black cap)	1×1.0mL
Antibody solution (blue cap)	1×5.5mL
HRP conjugate (red cap)	1×11mL
Substrate Reagent A (white cap)	1×6mL
Substrate Reagent B (black cap)	1×6mL
Stop Solution (yellow cap)	1×6mL
Concentrated Wash Buffer (20×)(white cap)	1×40mL

Concentrated Reconstitution Buffer(2×) (yellow cap)	1×50mL
Instruction	1
Adhesive Membrane	1
Sealed Bag	1

## 4 Materials Required but Not Supplied

**4.1 Equipment:** microplate reader, printer, grinder (for homogenizing solid samples), nitrogen evaporator, vortex mixer (for shake and mix), centrifuge, graduated transfer pipette, and balance with a division value of 0.01 g, constant temperature device(25°C);

**4.2 Micropipette:** single-channel (20-200μL and 100-1000μL), and multi-channel 300μL;

**4.3 Reagents:** Anhydrous Sodium Carbonate, Sodium Bicarbonate, n-Hexane, Ethyl Acetate.

## 5 Experimental preparation

Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use. This is a crucial step to ensure there is no precipitation in the reagents.

### 5.1 Notice Before Sample Processing:

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

### 5.2 Solution preparation:

#### Solution 1: 0.1 M Carbonate Buffer Solution

Dissolve 4.66g of anhydrous sodium carbonate and 0.5g of sodium bicarbonate in deionized water, mix thoroughly, and make up to a final volume of 500mL.

#### Solution 2: Reconstitution Buffer

Dilute the Concentrated Reconstitution Buffer (2×) 2 times with deionized water (Reconstitution Buffer (2×):

deionized water=1:1). It can be stored at 4 °C for one month.

### **Solution 3: Working Wash Buffer**

Dilute the concentrated wash buffer (20×) by a factor of 20 (Concentrated wash buffer/Deionized water= 1: 19).

### **5.3 Sample pretreatment steps:**

#### **5.3.1 Honey or tissue (fish, shrimp, poultry, liver, etc.) treatment.**

- 1) Weigh 3g  $\pm$  0.05g of honey or homogenized tissue sample, add 3mL of 0.1M carbonate buffer solution (Solution 1), and shake thoroughly for mixing.
- 2) Add 9mL of ethyl acetate, shake for 5 minutes at room temperature at 4000 rpm, and centrifuge for 5 minutes.
- 3) Transfer 3mL of the clear upper layer to a clean glass test tube and dry under nitrogen or air at 50-60°C.
- 4) Add 1mL of n-hexane, shake for 30 seconds, then add 0.5mL of Reconstitution Buffer (Solution 2) and shake for another 30 seconds. Centrifuge at 4000 rpm for 5 minutes at room temperature.
- 5) Remove the upper layer and take 100 $\mu$ L of the lower layer for analysis.

***Dilution times of the sample:0.5 Detection limits: 1.5ppb***

#### **5.3.2Egg treatment.**

- 1)Mix egg whites and yolks thoroughly.
- 2)Weigh 3g  $\pm$  0.05g of homogenized sample and place it into a 50mL centrifuge tube. Add 9mL of ethyl acetate, shake for 5 minutes, then centrifuge at 4000 rpm for 10 minutes at room temperature.
- 3)Transfer 3mL of the clear upper layer to a clean glass test tube and evaporate it to dryness under nitrogen or air at 50-60°C.

4)Add 2mL of n-hexane, shake for 5min, then add 1mL of Reconstitution Buffer (Solution 2) and shake for another 5min. Centrifuge at 4000 rpm for 5 minutes at room temperature.

5)Remove the upper layer and take 100 $\mu$ L of the lower layer for analysis.

***Dilution times of the sample:1 Detection limits: 3ppb***

#### **5.3.3 Milk treatment.**

- 1) Take 3mL of sample and place it into a 50mL centrifuge tube. Add 9mL of ethyl acetate, shake for 2 minutes, then centrifuge at 4000 rpm for 5 minutes at room temperature.
- 2) Transfer 3mL of the clear upper layer to a clean glass test tube and evaporate it to dryness under nitrogen or air at 50-60°C.
- 3) Add 2mL of n-hexane, shake for 30 seconds, then add 1 mL of Reconstitution Buffer (Solution 2) and shake for 2 min. Centrifuge at 4000 rpm for 5 minutes at room temperature.
- 4) Remove the upper layer and take 100 $\mu$ L of the lower layer for analysis.

***Dilution times of the sample:1 Detection limits: 1.5ppb***

## **6 ELISA procedure |**

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

**Step 1:** Number: Number the wells in sequence corresponding to the samples and standard, make 2-well parallel trials for each sample and standard, and record

their locations.

**Step 2:** Sample Incubation: Add 100 $\mu$ L of standard or sample into each numbered well, then add 50 $\mu$ L of antibody solution into each well. Finally, cover the Microtiter Plate with the adhesive membrane, shake gently by hand (or use a microplate shaker) for 5s and incubate for 30 min at 25°C in the dark.

**Step 3:** Washing: Uncover the adhesive membrane carefully, remove the liquid, pipette 350 $\mu$ L of Working Wash Buffer (Solution 3) to every well, let stand for 30 seconds then drain, repeat 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after tapping dry can be punctured with a clean pipette tip).

**Step 4:** Enzyme Incubation: Add 100 $\mu$ L of HRP conjugate into each well. Then cover the Microtiter Plate with the adhesive membrane, incubate for 30 min at 25°C in the dark.

**Step 5:** Washing: Same as step 3.

**Step 6:** Color: Add 50 $\mu$ L of Substrate Reagent A to each well. Then add 50 $\mu$ L of Substrate Reagent B per well. Shake gently by hand (or use a microplate shaker) for 5s, and allow to react for 15min at 25°C in the dark. (The reaction can be extended appropriately if the blue color is too pale.)

**Step 7:** Stop the reaction: Pipette 50 $\mu$ L of Stop Solution to each well, and shake gently by hand (or use a microplate shaker). The reaction would be stopped.

**Step 8:** Calculate: Determine the Optical Density (OD value; absorbance value) at 450nm (Reference wavelength 630nm) with a microplate reader. Finish this step within 10min after stop the reaction.

## 7 Interpretation of result |

### 7.1 Calculate the percentage of absorbance value

Percentage of absorbance value(%)=  $\frac{A}{A0} \times 100\%$

A—the average OD value of the sample or standard;

A0—the average OD value of the 0ppb standard.

It is used to calculate the percentage absorbance of a standard or sample.

### 7.2 Draw the standard curve and calculate

Take absorbance percentage(A/A0) of standards as Y-axis and the corresponding log of standards concentration (ppb) as X-axis.

Draw the standard semi-log curves with X-axis and Y-axis.

Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve. **Last, the resulting concentration values multiplied by the corresponding dilution times is the actual concentration of NMZ of samples.**

If professional analysis software of the kit is used for calculation, it is more convenient for accurate and rapid analysis of a large number of samples.

## 8 Attention |

8.1 Before test, the reagents and samples should be balanced to room temperature (25°C). If below 25°C, it will lead to all the standard OD value on the low side.

8.2 In the washing process, dry wells may result in non-linear standard curves and undesirable reproducibility. Therefore, proceed to the next step immediately after washing.

8.3 Please mix the contents within the wells uniformly and wash the plate thoroughly. The reproducibility is

largely determined by consistency of washing step.

8.4 During the incubation, cover microplates with adhesive membrane to avoid light.

8.5 Do not use kits that are overdue. Do not mix reagents with those from other lots.

8.6 Substrate Reagent A/B is colorless. If not, please discard.

8.7 If absorbance value of 0ppb is below 0.5 (A450nm< 0.5), it means that the reagent may be metamorphic.

8.8 Stop solution is corrosives, please avoid contact with skin.

8.9 As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.

8.10 For the mentioned sample, fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.

8.11 The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS can be used for quantitative confirmation.

## 9 Storage conditions |

The kit shall be stored at 2-8 °C. Avoid freezing.

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