# MNZ (Metronidazole) ELISA Kit

Catalog No: E-FS-E151

96T/96T*3

| | |
|---|---|
| **Version Number:** | V1.3 |
| **Replace version:** | V1.2 |
| **Revision Date:** | 2025.05.26 |

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.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

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 Competitive-ELISA as the method for the quantitative detection. It can detect Metronidazole (MNZ) in samples, such as muscle, honey, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate provided in this kit has been pre-coated with coupled antigen. During the reaction, MNZ in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti- MNZ antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and substrate reagent is for color development. There is a negative correlation between the OD value of samples and the concentration of MNZ. The concentration of MNZ in the samples can be calculated by comparing the OD of the samples to the standard curve.

## Technical indicator

**Reaction mode** (Incubation time and temperature)**:** 4℃; 25℃; 80 min, 10 min, 15-20 min.
**Detection limit:** Chicken, Duck, Pork, Honey---0.5 ppb; Milk---1 ppb; Egg---0.3 ppb
**Cross-reactivity:** Metronidazole (MNZ) ---100%; Dimetridazole (DMZ) ---150%
**Sample recovery rate:** 90%±30%

## Kits components

| Item | Specifications |
|---|---|
| ELISA Microtiter plate | 96 wells |
| Standard Liquid | 1.5 mL each (ppb=ng/mL=ng/g)<br>(0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb) |
| HRP Conjugate | 12 mL |
| Antibody Working Solution | 7 mL |
| Substrate Reagent A | 7 mL |
| Substrate Reagent B | 7 mL |
| Stop Solution | 7 mL |
| 20×Concentrated Wash Buffer | 25 mL |
| Sample Diluent | 50 mL |
| Plate Sealer | 3 pieces |
| Sealed Bag | 1 piece |
| Manual | 1 copy |

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

## Other materials required but not supplied

**Instruments:** Micro-plate reader, Printer, Homogenizer, Nitrogen evaporators, Oscillators, Vortex mixer, Graduated pipette, Balance (sensibility 0.01 g), Water bath.

**Micropipette:** Single channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).

**Reagents:** NaOH, HCl, $H_2SO_4$, Ethyl Acetate, N-hexane.

## Notes

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate 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. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E151. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E151 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.5 unit (A450nm < 0.5), it indicates the reagent be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. **For 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.**
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, etc. can be used for quantitative confirmation.

## Storage and expiry date

Store the kit at 2-8℃. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8℃.

**Expiry date:** expiration date is on the packing box.

## Experimental preparation

Restore all reagents and samples to room temperature (25℃) 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**

   *Please prepare solution according to the number of samples. Don't use up all components at once!*

   Solution 1: 0.3 M NaOH

   Dissolve 2.4 g of **NaOH** to 200 mL with deionized water, mix fully.

   Solution 2: 0.1 M HCl

   Take 8.33 mL of **HCl** and dilute it to 1 L with deionized water.

   Solution 3: 2 M $H_2SO_4$

   Slowly inject 10.65 mL of concentrated sulfuric acid into 70 mL of deionized water and make up to 100 mL with deionized water.

   Solution 4: Wash Buffer

   Dilute **20×Concentrated Wash Buffer** with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19).

   Solution 5: Honey dilution

   Dilute **Sample Diluent** with deionized water. (Sample Diluent (V): Deionized water (V) = 1:1).

3. **Sample pretreatment procedure**

**3.1 Pretreatment of Chicken, Duck, Pork, Eggs sample:**

(1) Remove fat from sample. Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 2±0.05 g of homogenate sample to 50mL centrifuge tube, add 2 mL of **0.3 M NaOH** (Solution 1), vortex for 30s.

(3) Add 6 mL of **Ethyl acetate**, vortex for 2 min, and centrifuge at 4000 r/min for 10 min at room temperature.

(4) Remove 3 mL of clear supernatant into a clear tube, dry at 55-60℃ in nitrogen evaporators or water bath (Please do it in a ventilated environment.).

(5) Add 2 mL of **N-hexane**, then add 0.5 mL of **Sample Diluent**, vortex for 30 s, centrifuge at 4000 r/min for 5 min at room temperature.

(6) Remove the upper N-hexane and the impurities in the middle layer; take 50 µL of lower liquid for analysis.

**Note: Sample dilution factor: 0.5,** **detection limit:** Chicken, Duck, Pork ---0.5 ppb **;** Egg---0.3 ppb

**3.2 Pretreatment of honey sample:**

(1) Weigh 2±0.05 g of honey sample into a 50 mL centrifuge tube. ( Note: If the honey sample crystallizes, place it in a 60℃ water bath for 30 minutes to allow the crystals to dissolve before proceeding with the operation.)

(2) Add 10 mL of **0.1 M HCl** (Solution 2) and 5 mL of **Ethyl acetate** in sequence and stir vigorously for 1 min, centrifuge at 4000 r/min for 10 min at room temperature.

(3) Take 6 mL of the lower layer liquid into a clean 50 mL centrifuge tube.

(4) Add 4 mL of **0.3 M NaOH** (Solution 1) and 6 mL of **Ethyl acetate** in sequence and stir vigorously for 1 minute, centrifuge at 4000 r/min for 10 min at room temperature.

(5) Remove 3 mL of clear supernatant into a clear tube, dry at 55-60℃ in nitrogen evaporators or water bath (Please do it in a ventilated environment.).

(6) Add 2 mL of **N-hexane** and 0.5 mL of **Honey dilution** (Solution 5), and stir thoroughly for 30 s, centrifuge at 4000 r/min for 5 min at room temperature.

(7) Remove the upper N-hexane and the impurities in the middle layer; take 50 µL of lower liquid for analysis.

**Note: Sample dilution factor: 1, detection limit: 0.5 ppb**

**3.3 Pretreatment of milk sample:**

(1) Weigh 1 mL of homogenate milk sample to a centrifuge tube, add 2 µL of **2 M $H_2SO_4$**(Solution 3), vortex for 1 min, and centrifuge at 4000 r/min for 5 min at room temperature.

(2) Remove the upper fat layer. Take 50 µL of the upper clear liquid and place it in 450 µL of the **sample dilution**, and vortex it fully for 30 s.

(3) Take 50 µL of lower liquid for analysis.

**Note: Sample dilution factor: 10, detection limit: 1 ppb**

## Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** number the sample and standard in order (multiple wells), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate.**
2. **Add Sample:** add 50 µL of **Standard or Sample** per well, then add 50 µL of **Antibody Working Solution**, cover the plate with plate sealer, oscillate for 5 s gently to mix thoroughly, incubate for 80 min at 4℃ in shading light. (Note: The **Antibody Working Solution** should be stored at 4℃ and does not need to be warmed up. Take it out and add samples when needed!)
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 260 µL of **Wash Buffer** (Solution 4) to each well and wash. Repeat wash procedure for 4 times, 30 s 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. **HRP Conjugate:** add 100 µL of **HRP Conjugate** to each well, incubate for 10 min at 25℃ in

shading light.

5. **Wash:** repeat step 3.
6. **Color Development:** add 100 μL of **Substrate Reagent A** and **Substrate Reagent B** mixture. (**Substrate Reagent A** and **Substrate Reagent B** are mixed 1:1 according to volume, must be fully mixed, the mixture is used within 5 minutes, avoid the use of metal container, avoid stirring reagents.) Gently oscillate for 5 s to mix thoroughly. Incubate 25℃ for 15-20 min at in shading light.
7. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 10 min 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 sample to 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 analysis software for accurate and fast analysis on a large number of samples.

**Metronidazole** (E-FS-E151) **Standard Curve**