MNZ (Metronidazole) ELISA Kit

Catalog No: E-FS-E011

96T/96T*3

Version Number:	V1.2
Replace version:	V1.1
Revision Date:	2024.03.14

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
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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 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)**:** 25℃; 30 min, 30 min, 15 min.
Detection limit: Muscle---1.5 ppb; Honey, Milk---1.5 ppb; Egg---3 ppb
Cross-reactivity: Metronidazole (MNZ) ---100%; Dimetridazole (DMZ) ---68%
Sample recovery rate: 90%±10%

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0 ppb, 1.5 ppb, 3.0 ppb, 6.0 ppb, 12.0 ppb, 24.0 ppb)
HRP Conjugate	11 mL
Antibody Working Solution	5.5 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20×Concentrated Wash Buffer	40 mL
2×Reconstitution Buffer	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: Na_2CO_3, $NaHCO_3$, N-hexane, Ethyl Acetate.

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-E011. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E011 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: 0.1 M Phosphate Buffer Solution *(for muscle, honey sample*)

Dissolve 4.66 g of **Na_2CO_3** and 0.5 g of **$NaHCO_3$** to 500 mL with deionized water, mix fully, pH 10.6.

Solution 2: Reconstitution Buffer

Dilute the **2×Reconstitution Buffer** with deionized water. (2×Reconstitution Buffer (V): Deionized water (V) = 1:1).

Solution 3: Wash Buffer

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

3. Sample pretreatment procedure

3.1 Pretreatment of muscle, honey sample:

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

(2) Weigh 3±0.05 g of homogenate sample to centrifuge tube, add 3 mL of **0.1 M Phosphate Buffer Solution** (Solution 1), vortex until sample dissolves completely.

(3) Add 9 mL of **Ethyl acetate**, vortex for 5 min, and centrifuge at 4000 r/min for 5 min at room temperature.

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

(5) Add 1 mL of **N-hexane**, vortex for 30 s add 0.5 mL of **Reconstitution Buffer** (Solution 2), vortex for 30 s, centrifuge at 4000 r/min for 5 min at room temperature.

(6) Remove upper organic liquid, take 100 μL of lower liquid for analysis.

Note: Sample dilution factor: 0.5, detection limit: 1.5 ppb

3.2 Pretreatment of egg sample:

(1) Weigh 3±0.05 g of homogenate sample to 50 mL centrifuge tube, add 9 mL of **Ethyl acetate**, vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature.

(2) Remove 3 mL of clear organic supernatant into a clear glass tube, dry at 55-60℃ in nitrogen evaporators or water bath. Add 2 mL of **N-hexane**, vortex for 5 min add 1 mL of **Reconstitution Buffer** (Solution 2), vortex for 5 min, centrifuge at 4000 r/min for 5 min at room temperature.

(3) Remove upper organic liquid, take 100 μL of lower liquid for analysis.

Note: Sample dilution factor: 1, detection limit: 3 ppb

3.3 Pretreatment of milk sample:

(1) Weigh 3 mL of homogenate milk sample to 50 mL centrifuge tube, add 9 mL of **Ethyl acetate**, vortex for 2 min, and centrifuge at 4000 r/min for 5 min at room temperature.

(2) Remove 3 mL of clear organic supernatant into a clear glass tube, dry at 55-60℃ in nitrogen evaporators or water bath. Add 2 mL of **N-hexane**, vortex for 30 s, add 1 mL of **Reconstitution Buffer** (Solution 2), vortex for 2 min, centrifuge at 4000 r/min for 5 min at room temperature.

(3) Remove upper organic liquid, take 100 μL of lower liquid for analysis.

Note: Sample dilution factor: 1, detection limit: 1.5 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 100 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 30 min at 25℃ in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 μL of **Wash Buffer** (Solution 3) to each well and wash. Repeat wash procedure for 5 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 30 min at 25℃ in shading light.
5. **Wash:** repeat step 3.
6. **Color Development:** add 50 μL of **Substrate Reagent A** to each well, and then add 50 μL of **Substrate Reagent B**. Gently oscillate for 5 s to mix thoroughly. Incubate shading light for 15 min at 25℃ in shading light (If the blue color is too shallow, can extend the incubation time properly).
7. **Stop Reaction:** add 50 μL of **Stop Solution** to each well, oscillate gently to mix thoroughly.
8. **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-E011) **Standard Curve**