Elabscience®

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Metronidazole (MNZ) ELISA Kit

Catalog No: E-FS-E011

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.

Phone: 240-252-7368(USA) 240-252-7376(USA)
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Test principle

This kit uses Indirect-Competitive-ELISA as the method. It can detect Metronidazole (MNZ) in samples, such as tissue and honey, 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 MNZ. During the reaction, MNZ in the samples or standard competes with MNZ on the solid phase supporter for sites of MNZ 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 MNZ. The concentration of MNZ in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.5 ppb (ng/mL)
Reaction mode: 25℃, 30 min～30 min～15 min
Detection limit: Tissue (chicken, duck/Liver, fish, Shrimp, etc.) ---0.25 ppb, Honey---0.25 ppb
Cross-reactivity: Metronidazole (MNZ) ---100%, Dimetridazole (DMZ) ---68%
Sample recovery rate: Fish/shrimp/poultry/liver---90%±10%, Honey---90%±10%

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Liquid (black cap)	1 mL each (0 ppb, 0.5 ppb, 1.5 ppb, 4.5 ppb,13.5 ppb, 40.5 ppb)
High Concentrated Standard (100 ppb)	1 mL
HRP Conjugate (red cap)	11 mL
Antibody Working Solution (blue cap)	5.5 mL
Substrate Reagent A (white cap)	6 mL
Substrate Reagent B (black cap)	6 mL
Stop Solution (yellow cap)	6 mL
20×Concentrated Wash Buffer (white cap)	40 mL
2×Reconstitution Buffer (yellow cap)	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Other supplies required

Instruments: Micro-plate reader, Printer, Homogenizer, Nitrogen Evaporators, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g), Incubator.

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

Reagents: NaOH, Anhydrous Sodium Sulfate, 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 use disposable pipette tips to avoid cross-contamination during the experiment.

2. Solution preparation

Solution 1:0.1 M Buffer Bicarbonate.

Dissolve 4.66 g of anhydrous sodium sulfate and 0.5 g of sodium bicarbonate with 500 mL of deionized water，pH 10.6.

Solution 2: 2 M NaOH Solution

Dissolve 40 g of solid NaOH with 500 mL of deionized water.

Solution 3: Wash Buffer

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

Solution 4: Redissolve Solution

Dilute the 2×Reconstitution Buffer with deionized water. (2×Reconstitution Buffer (V): Deionized water (V)=1:1) .This 1× Reconstitution solution can be store at 4℃ for a month.

3. Sample pretreatment procedure

Pretreatment of Tissue (chicken, duck/liver, fish, shrimp, etc.) and honey:

(1) Weigh 3 g of sample, add 3 mL of 0.1 M buffer bicarbonate, oscillate until honey dissolves completely;

(2) Add 9 mL of ethyl acetate, oscillate for 5 min, centrifuge at 4000 r/min for 5 min at room temperature;

(3) Remove 6 mL of upper organic phase into another EP tube, add 2 mL of ethyl acetate and 2 mL of 2 M NaOH solution, oscillate for 5 min, centrifuge at 4000 r/min for 5 min at room temperature;

(4) Remove 4 mL of clear organic supernatant into a clear glass tube, blow-dry in nitrogen or air at 30～40℃.

(5) Add 1 mL of n-hexane, swirling for 30 sec add 0.5 mL of reconstituted solution，swirling for 30 sec，centrifuge at 4000 r/min for 5 min at room temperature；

(6) Remove upper organic liquid, take 500 μL of sub-layer liquid for analysis.

Note: Sample dilution factor: 0.5, minimum detection dose: 0.25 ppb

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.

1. **Number:** number the sample and standard in order (multiple wells), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50 μL of Standard or Sample per well, then add 50 μL of antibody working solution, cover the plate with sealer we provided, oscillate for 5 sec gently to mix thoroughly, incubate for 30 min at 37℃.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 250 μL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 sec 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 37℃.
5. **Wash:** repeat step 3.
6. **Color Development:** add 50 μL of substrate solution A to each well, and then add 50 μL of substrate solution B. Gently oscillate for 5 sec to mix thoroughly. Incubate shading light for 15min at 37℃. (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 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 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 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 below 25℃.
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)＜0.5 unit($A_{450\ nm}$＜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.

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