



## Metronidazole ELISA Test Kit (Honey)

Catalog No. LSY-10048

### 1. Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Metronidazole in the sample. The coupling antigens are pre-coated on the micro-well stripes. The Metronidazole in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-Metronidazole antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Metronidazole in it. This value is compared to the standard curve and the Metronidazole concentration is subsequently obtained.

### 2. Technical specifications

**Sensitivity:** 0.05ppb

**Detection limit**

Honey: about 0.1ppb

Note: ppb= ng/ml or ng/g

**Cross-reaction rate**

Metronidazole.....	100%
Ornidazole .....	83%
Secnidazole .....	110%
Metronidazole-OH .....	<1%
Tinidazole .....	<1%
Dimetridazole .....	<1%

**Recovery rate:** 90±30%

**Coefficient of variation for same plate:** <5%

**Coefficient of variation for different plate:** < 10%

### 3. Components

- 1) Micro-well strips: 12 strips with 8 removable wells each
- 2) 6× standard solution (1mL each): 0ppb, 0.05ppb, 0.15ppb, 0.45ppb, 1.35ppb and 4.05ppb
- 3) Enzyme conjugate (12 mL)..... 1 bottle
- 4) Antibody working solution (7mL)..... 1 bottle
- 5) Substrate A solution (7 mL)..... 1 bottle
- 6) Substrate B solution (7 mL) .....
- 7) Stop solution (7 mL)..... 1 bottle
- 8) 20× concentrated washing buffer (30 mL) .....
- 9) Redissolving solution (50 mL)..... 1 bottle

### 4. Materials required but not provided



- 1) **Equipments:** microplate reader (450nm, 630nm), printer, nitrogen-drying device, vortex, shaker, centrifuge (3000g and above), measuring pipets, balance (a reciprocal sensibility of 0.01 g), incubator (4°C, 25°C), water bath, timer;
- 2) **Micropipettors:** single-channel 20-200 µL, 100-1000 µL, and eight-channel 30~300 µl;
- 3) **Reagents (AR):** Na<sub>2</sub>HPO<sub>4</sub>•12H<sub>2</sub>O, NaH<sub>2</sub>PO<sub>4</sub>•2H<sub>2</sub>O, Ethyl acetate, Hexane, deionized water.

## 5. Sample pre-treatment

### Instructions

The following points must be dealt with before the pre-treatment of any kind of sample:

- 1) Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents;
- 2) Before the experiment, each experimental equipment must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

### *Solution preparation before sample pre-treatment:*

- 1) **Washing buffer:** 1 part 20× concentrated washing buffer + 19 parts deionized water.
- 2) **0.2M Phosphate buffer:** weigh 25.8g Na<sub>2</sub>HPO<sub>4</sub>•12H<sub>2</sub>O, 4.350g NaH<sub>2</sub>PO<sub>4</sub>•2H<sub>2</sub>O, add 500ml deionized water to dissolve completely.

### 5.1 Sample preparation

#### Honey

- 1) Weigh 2.0± 0.05g honey sample into 50ml plastic centrifuge tube;
- 2) Add 2ml **0.2M Phosphate buffer**, then add 8ml Ethyl acetate, shake strongly for 5min (or use vortex for 3min) to make sample contact with organic phase completely;
- 3) Centrifuge at above 3000 g at room temperature for 5min;
- 4) Transfer 4ml up-layer clear liquid into 5ml centrifuge tube (**note: do not take down-layer water phase**), blow to dry in 50-60°C water bath;
- 5) Add 1ml Hexane and 0.5ml Redissolving solution accordingly, shake strongly for 2min (or use vortex for 1min);
- 6) Centrifuge at above 3000 g at room temperature for 5min;
- 7) Discard up-layer organic phase, take 50ul down-layer liquid to test.

**Fold of dilution of the sample: 0.5**

## 6. ELISA procedures

### 6.1 Instructions

- 1) Bring all reagents and micro-well strips to the room temperature (20-25 °C) before use;
- 2) Return all reagents to 2-8 °C immediately after use;
- 3) The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in ELISA the procedures;
- 4) For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

### 6.2 Operation procedures

1. Bring test kit to the room temperature (20-25 °C) for at least 30 min, note that each reagent must be shaken to mix evenly before use, put the required micro-well strips into plate frames. Re-sealed the unused microplate, store at 2-8 °C, not frozen.



2. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate, record their positions.
3. Add 50  $\mu\text{L}$  of the sample or standard solution into separate duplicate wells; then add 50  $\mu\text{L}$  of the antibody working solution into each well, mix gently by shaking the plate manually. Seal the microplate with the cover membrane, and **incubate at 4  $^{\circ}\text{C}$  for 60min in dark.**
4. Pour liquid out of microwell, add 250  $\mu\text{L}$ /well of washing buffer to wash microplate for 15-30 s, then pour washing buffer out of microwell, add washing buffer to wash in this way for 3-4 times, then take out and flap to dry with absorbent paper.
5. Add 100ul enzyme conjugate(do not put the microplate at room temperature for long time after washing step to avoid dryness of microplate lead to result's difference ), mix gently by shaking the plate manually. Seal the microplate with the cover membrane, and **incubate at 25  $^{\circ}\text{C}$  for 20min in dark.** Washing as step 4.
6. Coloration: add 100ul mixture of substrate A solution and substrate B solution into each well (Note: mix substrate A solution and substrate B solution at 1:1, use the mixture in 10min, do not use metal to contain or stir, to avoid substrate invalid). Mix gently by shaking the plate manually, seal the microplate with the cover membrane then **incubate at 25  $^{\circ}\text{C}$  for 15 min at dark for coloration.**
7. Determination: add 50  $\mu\text{L}$  of the stop solution into each well. Mix gently by shaking the plate manually. Stop successfully when substrate color from blue to yellow. Recommend to read the OD value at the dual-wavelength 450/630 nm within 5 minutes.

## 7. Result judgment

There are two methods to judge the results: the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the Metronidazole concentration.

### 7.1 Qualitative determination

The concentration range (ng/mL) of Metronidazole can be obtained from comparing the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample I is 0.3, and that of the sample II is 1.0, the OD value of standard solutions is: 2.243 for 0ppb, 1.816 for 0.05ppb, 1.415 for 0.15ppb, 0.74 for 0.45ppb, 0.313 for 1.35ppb, 0.155 for 4.05ppb, accordingly the concentration range of the sample I is 1.35 to 4.05ppb, and that of the sample II is 0.05 to 0.45ppb.

### 7.2 Quantitative determination

The mean values of the absorbance values is obtained for the average OD value (B) of the sample and the standard solution divided by the OD value ( $B_0$ ) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

$$\text{Percentage of absorbance value} = \frac{B}{B_0} \times 100\%$$

B—the average OD value of the sample or the standard solution

$B_0$ —the average OD value of the 0 ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solution and the semilogarithm values of the Metronidazole standard solution (ng/mL) as Y- and X-axis, respectively.



Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the Metronidazole concentration in the sample. Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software).

## 8. Precautions

1. The room temperature below 25 °C or the temperature of the reagents and the samples being not returned to the room temperature (20-25 °C) will lead to a lower standard OD value.
2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.
3. Mix evenly, otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lots to use.
6. Put the unused microplate into an auto-sealing bag to re-seal it. The standard solution and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.
7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the standard solution 1(0 ppb) of less than 0.5 indicates its degeneration.

## 9. Storage and expiry date

**Storage:** store at 2-8 °C, not frozen.

**Expiry date:** 12 months; date of production is on box.

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