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Metronidazole ELISA Kit

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(Catalog # E4948-100, 96 assays, Store at 4°C)

I. Introduction:

Metronidazole is an antibacterial and antiprotozoal agent that belongs to the class of nitroimidazoles. It is used to treat parasitic infections such as trichomoniasis, giardiasis, gastrointestinal infections, and bacterial infections in humans. Although the exact mechanism is unknown, it is believed that an intermediate formed by the reduction of metronidazole by anaerobic organisms binds to the DNA and electron-transport proteins of these organisms and inhibits nucleic acid synthesis. The antibiotic has been demonstrated to cause cancer in mice and rats when exposed to high concentrations for a long period. Similar risks may be associated with humans. Due to its carcinogenic properties, this drug is prohibited from extra-label use in food animals. BioVision's Metronidazole ELISA kit is used to quantitatively measure Metronidazole in muscle tissue, honey, and egg samples. The kit is based on the Competitive ELISA principle. Samples and standards are added to the microwell plate that is pre-coated with an antigen and competes for binding to the anti-Metronidazole antibody. The HRP conjugate is added to each well and any unattached conjugates are washed off using Wash Buffer. The HRP enzymatic reaction is detected by the addition of substrate reagents. Finally, the reaction is terminated with an acidic stop solution. The color developed is inversely proportional to the concentration of Metronidazole in the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Metronidazole
Detection Limit: 1.5ppb for muscle tissue, 1.5ppb for honey, 3ppb for egg
Sensitivity: 1.5ppb
Cross reaction: Metronidazole (MNZ) 100%, Dimetridazole (DMZ) 68%

III. Sample Type:

Tissue, Honey, Egg

IV. Kit Contents:

Components	E4948-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4948-100-1
Standard (S0 – S5)	1 ml X 6	E4948-100-2
HRP Conjugate	11 ml	E4948-100-3
Antibody working solution	5.5 ml	E4948-100-4
Substrate A	6 ml	E4948-100-5
Substrate B	6 ml	E4948-100-6
Stop Solution	6 ml	E4948-100-7
Wash Buffer (20X)	40 ml	E4948-100-8
Reconstitution Buffer (2X)	50 ml	E4948-100-9
Plate Sealer	3	E4948-100-10

V. User Supplied Reagents and Equipment:

- Chemicals: deionized water, Na_2CO_3, $NaHCO_3$, n-Hexane, Ethyl acetate
- Microplate reader, Nitrogen evaporator
- Clean eppendorf tubes and graduated cylinders for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer (1X):** Dilute 1 part of **Wash buffer (20X)** with 19 parts of deionized water. Prepare quantity as needed.

2. **Standards Preparation:** Ready-to-use standards provided as follows

Standards	S0	S1	S2	S3	S4	S5
Conc. (ppb)	0	1.5	3	6	12	24

3. **Sample Preparation:**
Note: The prepared sample maybe stored for up to one day at 2-8°C.

Sample pre-treatment: The following method must be used for pre-treatment of any kind of sample:
Note: Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.

FOR RESEARCH USE ONLY! Not to be used on humans.

Solution preparation before sample pre-treatment:

1) **Reconstitution Buffer (1X):** Dilute 1 part of **Reconstitution Buffer (2X)** with 1 part of deionized water.
2) **0.1M CB solution (pH 10.6):** Take 4.66 grams of Na_2CO_3 and 0.5 grams of $NaHCO_3$ and make up the volume to 500 ml with deionized water

Sample Preparation and pre-treatment (for muscle and honey samples):

Detection limit: 1.5ppb

- Take 3 grams of the homogenized sample into a centrifuge tube. Add 3 ml of **0.1M CB solution (pH 10.6).** Mix until sample dissolves completely.
- Add 9 ml **Ethyl acetate**, mix for 5 mins, and centrifuge at 4000 r/min for 5 mins at room temperature (RT).
- Take 5 ml of the clear organic supernatant in a new glass tube and dry with nitrogen evaporator or water bath at 40 °C.
- After drying, add 1 ml of **n-Hexane**, mix for 30 secs and then add 0.5 ml of **Reconstitution Buffer (1X)**. Mix for 30 secs and then centrifuge at 4000 r/min for 5 mins at RT.
- Discard the upper organic layer. Take 50 µl of the lower liquid for the analysis.
- **Fold of dilution of the sample: 0.5**

Sample Preparation and pre-treatment (for egg samples):

Detection limit: 3ppb

- Take 3 grams of the homogenized sample into a centrifuge tube. Add 9 ml **Ethyl acetate**, mix for 5 mins, and centrifuge at 4000 r/min for 5 mins at room temperature (RT).
- Take 3 ml of the clear organic supernatant in a new glass tube and dry with nitrogen evaporator or water bath at 40 °C.
- After drying, add 2 ml of **n-Hexane**, mix for 5 mins and then add 1 ml of **Reconstitution Buffer (1X)**. Mix for 5 mins and then centrifuge at 4000 r/min for 5 mins at RT.
- Discard the upper organic layer. Take 50 µl of the lower liquid for the analysis.
- **Fold of dilution of the sample: 1**

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Add 100 µl of the **sample or standards** to separate duplicate wells. Add 50 µl of the **Antibody working solution** into each well. Mix gently for 5 secs by shaking the plate manually, seal the microplate with the plate sealer, and incubate in dark at 25 ^{0}C for 30 mins.
2. Remove the plate sealer carefully, aspirate liquid out of microwells, and add 300 µl of **Wash Buffer (1X)** to each well. Wash for 30 secs, and then discard the buffer. Repeat the washing step five times. After the final wash step, tap to dry (if there are the bubbles after tapping, remove them with the clean tips).
3. Add 100 µl **HRP Conjugate** in each well, and then incubate the plate at 25 ^{0}C for 30 mins in the dark.
4. Repeat washing as mentioned in **step 2**.
5. Add 50 µl of the **Substrate A** and then add 50 µl of the **Substrate B** into each well. Mix gently for 5 secs by shaking the plate manually, and incubate at 25 ^{0}C for 15 mins in dark.
6. Add 50 µl of the **Stop Solution** into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (Recommend reading the OD value at the wavelength 450 nm within 5 mins).

IX. Calculation:

- **Quantitative determination**

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

Absorbance Value (%) = B/B_0 X 100%

B: The average absorbance value of the sample or standard

B_0: The average absorbance value of the 0 ppb standard

To draw a standard curve: Plot the absorbance value of standards as y-axis, logarithmic of the concentration of the Trimethoprim standards solution (ppb) as x-axis. The Trimethoprim concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

X. Related Products:

Sulfamethazine ELISA Kit (E4778)
Enrofloxacin (ENR) ELISA Kit (E4277)
Aflatoxin M1 ELISA Kit (E4566)
Quinolone ELISA Kit (E4530)
Tylosin ELISA Kit (E4779)