UC Biodevices

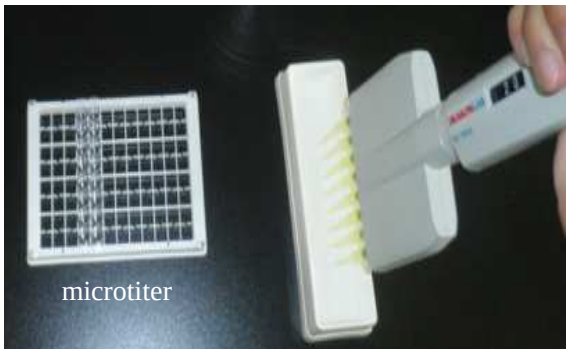
ELISA Kit for Detection of Enrofloxacin

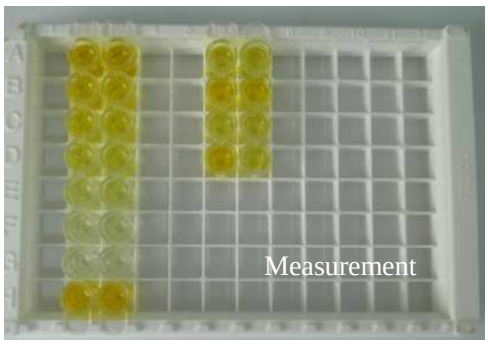
(Product Number: 5501E210)

（Enrofloxacin has potential carcinogenicity）

INSTRUCTION MANUAL

(v. 1.00)





1. Introduction

Enrofloxacin belongs to the quinolones family. It is a synthetic antibiotic which acts by inhibition of bacterial DNA-gyrase. Enrofloxacin is currently FDA-approved for treatment of individual pets and domestic animals in the United States. It is also used for therapy, prevention and growth promotion. For its leading to drug resistance and the potential carcinogenicity, the maximum residue limit of Enrofloxacin inside animal body has been prescribed in the EU, Japan and China.

This manual establishes procedures for determining enrofloxacin in meat and honey. The test is a competitive direct ELISA that provides exact concentrations in parts per billion (ppb). Free toxin in the samples and controls competes with enzyme-labeled toxin (conjugate) for the antibody binding sites. After a wash step, substrate reacts with the bound enzyme conjugate to produce blue color.

Assay Sensitivity, Precision, Accuracy

1) Sensitivity: 0.2ppb； 1ppb in meat; 2ppb in honey
2) Precision： <10-20%
3) Accuracy： 105％ average recovery rate

2. Kit Contents

1) Microtiter plate (8 wells×12) precoated with antibodies to mouse IgG.
2) 5×Ennofloxacin standard solutions (1ml/each):0ng/ml, 2.0ng/ml, 5.0ng/ml, 20.0ng/ml, 50.0ng/ml, 100.0ng/ml

   Controls provided: 0ppb, 2.0ppb, 5.0ppb, 20.0ppb, 50.0ppb and 100.0ppb
3) Enzyme Conjugate(100×) (0.2ml)
4) Antibody (100×): Enrofloxacin (0.2ml)
5) Washing buffer(10×) (50ml)
6) Diluent buffer (50ml)
7) TMB (18ml)
8) Stopping solution (7ml)
9) Instruction Manual

Materials and reagents required but not provided:

1) 50, 100 and 200µl precision micropipette.

2) 50-200µl multichannel micropipette.
3) microtitre plate reader with 450nm filter.
4) methenyl trichloride and n-hexane
5) Acetonitrile - ACS grade or better.
5) 250ml graduated cylinder.
6) 125ml container.
7) Centrifuge（4000r/min）and vortex mixer
8) Timer

3. Preparation of Working Solutions

- **Enrofloxacin standard solutions**: ready to use.
- **100× Enzyme Conjugate**: dilute 100× with Antibody and Enzyme diluent buffer (1+99).
- **100×Antibody**: dilute 100× with Antibody and Enzyme diluent buffer (1+99).
- **washing buffer**: dilute 10× with distilled water (1+9).
- **TMB**: ready to use.
- **stopping solution**: ready to use.
- **0.1 M NaOH:** 0.4 g NaOH, dissolve in approximately 100 mL purified water.
- **Acetonitrile-0.1M NaOH:** 84ml acetonitrile add 16ml 0.1M NaOH dissolve in approximately.
- **pH 7.2 0.02M PBS:** 5.16g $Na_2HPO_4 \cdot 12H_2O$, 0.87g $NaH_2PO_4 \cdot 2H_2O$, dissolve in approximately 1000 mL purified water.
- **pH 7.2 0.1 M PBS:** 25.8g $Na_2HPO_4 \cdot 12H_2O$ 4.35g $NaH_2PO_4 \cdot 2H_2O$, dissolve in approximately 1000 mL purified water.

4. Preparation of Samples

Meat

a. Transfer 3 g of the homogenized sample into a centrifugal screw cap vial, add 9ml acetonitrile-0.1M NaOH buffer and shake for 30 min (with up-side-down shaker)
b. Centrifugation: 10 min/3000g / 15℃
c. Transfer 2 ml of the supermatant into a new centrifugal vial and add 2 ml 0.02M PBS buffer, 4 ml methenyl chloride, then shake vigorously for 10 min

d. Centrifugation: 10 min/3000g / 15℃

e. Transfer the methenyl chloride solution into a new centrifugal vial and evaporate to dryness at 50℃

f. Dissolve the residue in 1 ml n-hexane; add 1 ml Diluent buffer and vortex for 30 sec

g. Centrifugation: 10 min/4000 g/room temperature

h. Pour away the upper solution and use 50µl below solution per well in the assay

Honey

a. Transfer 1 gram honey into an extraction mixing jar.

b. Add 1 ml of 0.1 M PBS buffer and shake vigorously to dissolve the honey.

c. Add 8ml methenyl chloride and shake for 10 min (with up-side-down shaker)

d. Centrifugation: 10 min/4000g / room temperature

e. Transfer 4 ml of the methenyl chloride solution into a new centrifugal vial and evaporate to dryness at 50℃

f. Dissolve the residue in 2 ml Diluent buffer

g. Use 50µl per well in the assay

5. Immunoassay Procedure

a) Allow reagents, microwells, and sample extracts to reach room temperature prior to running the test.
b) Insert a sufficient number of wells into the microwell holder for all standards and samples to be tested.
c) Using a new pipette tip for each standard and sample, pipette 50µl of standards and prepared sample to separate wells.
d) Add 50µl of enzyme conjugate into each well.
e) Add 50µl of anti- enrofloxacin antibody into each well.
f) Incubate for 20 minutes at room temperature (37℃)
g) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.
h) Using a wash bottle, fill each well with washing buffer .Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 4 washes).

i) Add 150µl of TMB to each well. Incubate for 10 minutes at room temperature (37℃). Cover the wells with a paper towel to protect them from light sources.

j) Add 50µl of stop solution to each well.

k) Read results using a microwell reader with a 450 nm Thermo Labsystems.

6. Calculation

Divide the mean absorbance value of standards and samples (B) by the mean absorbance value of the Maximum Binding (Bo) and multiply by 100. Maximum binding is thus made equal to 100% and the absorbance values are quoted in percentages:

$$\frac{\text{absorbance standard (or sample)}}{\text{absorbance maximum binding}} \times 100 = B/Bo\ \%$$

7. Results

Since the samples have been diluted prior to assay, the concentrations in ng/ml read from the standard curve must be multiplied by the respective dilution factor to obtain the effective enrofloxacin concentration in samples expressed in ppb (ng/ml or ng/g). The dilution factors are 2 for Meat, 4 for honey.

8. Cautions

1) If ambient temperature is lower than 20℃ or the temperature of the reagents and standard solutions is less than the ambient temperature (e.g., 20- 25℃） when used, the testing results may have negative bias (lower than it should be).
2) If it takes too long time to dry the washed microtiter during the washing steps, the results may demonstrate nonlinear calibration plot and poor repeatability, thus once the microtiter is dried after the washing steps, the following step should be immediately implemented to prevent the undesirable occurrence.
3) The standard solutions and chromogenic solution contain light-sensitive substances, thus the solutions should not be exposed under direct light.
4) To gain excellent results, any solutions should be thoroughly mixed before added on microtiter

and the microtiter should be completely cleaned in between each step.

5) The stopping solution is a very strong acidic solution, so users should avoid the solution directly contacting with skin.
6) Neither use expired reagents, nor dilutes reagents or employs reagents produced by varied companies; otherwise, only gain less sensitive results.
7) Do not interchange individual reagents between kits of different lot numbers.
8) The kit should be stored at 2-8℃ but not be frozen. Place any unused microwells to their original foil bag and reseal them together with the desiccant provided.

9. Contact Information

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