Enrofloxacin (ENR) ELISA Diagnostic Kit

Introduction

Enrofloxacin (abbreviated as ENR in following text) is a member of Fluoroquinolone family, which is the second generation antibiotics of Quinolone, and often used as medicine for treating different kinds of human bacterial infections. In early days, this antibiotics family can be legally used on chicken and cattle in the United States. However, the abuse of the antibiotics for poultry disease treatment develops the Campylobacter, generating the food poison, has the resistance to the action of Fluoroquinolone. Recently, ENR residues have detected from the import and export cultivated eel in many countries. Accordingly, many countries have banned the Fluoroquinolone family.

Traditionally, ENR is determined commonly by HPLC and TLC techniques which are time-consuming and uneconomical. FLOGEN Enrofloxacin (ENR) ELISA Diagnostic Kit settles the disadvantages and further provides considerably advantages regarding sensitivity, detection limit, technical equipment and reproducibility. FLOGEN Enrofloxacin (ENR) ELISA Diagnostic Kit allows analyzing large amount of samples within a short period of time (90 minutes) with high sensitivity.

FLOGEN Enrofloxacin (ENR) ELISA Diagnostic Kit is design to exam the ENR remaining in serum and meat, the ENR detection limit can reach upto 3 ppb respectively.

Principle

The FLOGEN quantitative ENR ELISA kit is based on the specific immunochemical reaction between antigens and antibodies. It relies on the competition between ENR-HRPO conjugates and ENR residues in the sample to bind with the ENR antibodies immobilized on the microtiter wells. In the test procedure, the sample and the ENR-HRPO conjugates are added into the antibody-immobilized microtiter wells. If ENR is present in the sample, it will compete with the ENR-HRPO conjugates to bind with the limited amount of antibodies, which are immobilized on the microtiter wells. When a sufficient amount of ENR is present, ENR will saturate the antibodies. Thus the enzyme-substrate reaction will not generate and only light color shown, indicating a positive result. If there is a negative sample (0ppb), it will generate deep color considering the enzyme-substrate reaction.

Product Content

1. Microtiter well plate : 8 microtiter wells / strip, 12 strips / plate.
2. ENR standards (6 bottle) : 0 ppb, 0.3 ppb, 0.6 ppb, 1.2 ppb, 2.4 ppb, 4.8 ppb, 1.5 mL / bottle.
3. 10X Conc. Extraction diluent : 1 bottle, 40 mL / bottle.
4. 10X Conc. Washing solution : 1 bottle, 25 mL / bottle.
5. 100x ENR-enzyme conjugate : 1 bottle, 150 µL / bottle.
6. Substrate solution (TMB) : 1 bottle, 12 mL / bottle.
7. Stop solution : 1 bottle, 13 mL / bottle.

Operation Procedure

A. Precaution

1. Take out the kit from the refrigerator for more than 30 minutes before use to make sure the whole kit is back to room temperature. Mix every solution well before use.
2. Take out the required amount of microtiter well strips. The remaining strips should be put back into the aluminum bag, tape the closure, and store in the 4 ℃ refrigerator.

Dilute the 10X Conc. Extraction Diluent with 9 folds of double distilled water.(e.g., 10 mL 10X Conc. Extraction Diluent + 90 mL d2H2O)
Dilute the 10X Conc. Washing Solution with 9 folds of double distilled water.(e.g., 10 mL Conc. Washing Solution + 90 mL d2H2O )
Dilute the 100X Conc. ENR-enzyme Conjugate 100 folds of 1X Extraction Diluent.(e.g., 10 µL 100X ENR-enzyme Conjugate + 1 mL 1X Extraction Diluent )

Note: Please prepare the fresh 1X ENR-enzyme Conjugate based on the requirement each time.
B. Materials required but not provided

1. **Instruments**
   - (1) ELISA reader
   - (2) Centrifuge
   - (3) Shaker (Vortex)
   - (4) Mixer (Stomacher, Ultraturrax)
   - (5) Pipet aid
   - (6) 10µL, 100µL, 1000µL, 5000µL Pipetment, adjustable
   - (7) Multichannel pipette

C. Sample Preparation

1. **Serum (eel):**
   1. Add 0.1 mL of serum sample with 0.9 mL 1X Extraction Diluent to an eppendorf.
   2. Vortex for 1 minute.
   3. Centrifuge at 3000 rpm (>1700 g) for 10 minutes.
   4. Transfer 50 µL of supernatant for assay.
   
   **Sensitivity:** 3 ppb
   **Dilution Factor:** 10

2. **Meat (shrimp, eel):**
   1. Weigh 0.5 g of homogenized tissue and add 4.5 mL 1X Extraction Diluent to a 15 mL centrifuge tube.
   2. Vortex for 1 minute.
   3. Centrifuge at 3000 rpm (>1700 g) for 10 minutes.
   4. Transfer 50 µL of supernatant for assay.
   
   **Sensitivity:** 3 ppb
   **Dilution Factor:** 10

D. Operation procedure

1. Add 50 µL of the ENR Standards (0, 0.3, 0.6, 1.2, 2.4, 4.8 ppb) to the appropriate microtiter wells.
2. Add 50 µL of the prepared samples to the appropriate microtiter wells.
3. Add 100 µL of the 1X ENR-enzyme Conjugate to each well.
4. Mix by knocking the edge of the plate gently & incubate for 30 minutes at room temperature (19~25 °C) in the dark.
5. Dump the reaction solution out of the microtiter wells.
6. Add 1X Washing Solution to fill the microtiter wells then dump it out. Repeat this procedure 3 times.
7. Pat it dry after the final washing step.
8. Add 100 µL of the Substrate Solution to each microtiter well.
9. Mix by knocking the edge of the plate gently & incubate for 20 minutes at room temperature (19~25 °C) in the dark.
10. Add stop solution 100µL to each microtiter well.
11. Measure the absorbance at 450 nm. (ref. 630 or 650nm)

E. Test result interpretation

1. Divide the absorbance value of each standard and sample by the absorbance of the Maximum Binding (B0) (absorbance of 0 ppb standard) and multiply by 100, the Maximum Binding is thus made equal to 100% and the absorbance values are quoted in percentage:

   \[
   \frac{\text{absorbance standard (or sample)}}{\text{absorbance (0 ppb standard)}} = \frac{\text{B}}{\text{B0}} = 100 \%
   \]

2. Enter the calculated B/B0 values of each standard in a semi-logarithmic system of coordinates against the standard Enrofloxacin concentration, draw the standard curve. (Fig.1) Take the B/B0 values for each sample and interpolate the corresponding concentration from the calibration curve.

   For example: Divide the absorbance value of each sample by the absorbance of 0 ppb standard and multiply by 100 = X %, then the concentration = \(e^{(\frac{93.928-X}{23.723})}\) and multiply by the sample’s dilution factor (ppb). (Fig.1)
F. Detection limit
The lowest detection limit of the FLOGEN Enrofloxacin kit is about 0.3 ppb that means the OD value of this concentration is significantly different from the negative standard. (B/B₀ < 90%)

G. Standard Curve

\[
y = -25.723 \ln(x) + 53.928
\]

\[
R^2 = 0.994
\]

![Fig. 1: Calibration Curve for FLOGEN Enrofloxacin Kit](image)

H. Reproducibility
The precision within a series was determined from the result of six different experiments. The coefficient of variation (CV%) obtained for the standards absorbance are entered against the corresponding Enrofloxacin concentrations. The coefficient of variation is so low with respect to the whole range of the figure that a high reproducibility of the results is ensured.

![Fig. 2: Interassay Precision Profile for FLOGEN Enrofloxacin Kit](image)

I. Specificity
The specificity of Enrofloxacin ELISA was determined by analyzing the cross-reactivity of the corresponding substances.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrofloxacin</td>
<td>100</td>
</tr>
<tr>
<td>Ofloxacn</td>
<td>1</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>0.5</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.5</td>
</tr>
<tr>
<td>Sarafloxacin</td>
<td>0.1</td>
</tr>
<tr>
<td>Danofloxacin</td>
<td>0.1</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Oxolinic acid</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Flumequine</td>
<td>&lt; 0.1</td>
</tr>
</tbody>
</table>

J. Sensitivity

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sensitivity (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (eel)</td>
<td>3</td>
</tr>
<tr>
<td>Meat (shrimp, eel)</td>
<td>3</td>
</tr>
</tbody>
</table>

K. Recovery rate

<table>
<thead>
<tr>
<th>Sample (spike ENR)</th>
<th>Recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (3 ppb)</td>
<td>90–120</td>
</tr>
<tr>
<td>Meat (3 ppb)</td>
<td>80–130</td>
</tr>
</tbody>
</table>