Norfloxacin ELISA Kit
(Catalog # E4776-100; 96 assays, Storage at 4°C)

I. Introduction:
Norfloxacin is an antibiotic that belongs to the class of fluoroquinolone antibiotics. It is used to treat urinary tract infections, gynecological infections, inflammation of the prostate gland, gonorrhea and bladder infection. Norfloxacin is associated with a number of rare serious adverse reactions as well as spontaneous tendon ruptures and irreversible peripheral neuropathy. Tendon problems may manifest long after therapy had been completed and in severe cases may result in lifelong disabilities. Norfloxacin ELISA Kit is based on Competitive ELISA principle. The micro-plate provided in this kit has been pre-coated with Norfloxacin. During the reaction, Norfloxacin in the samples or standard competes with Norfloxacin coated on the plate for binding to the anti-Norfloxacin 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 Norfloxacin. The concentration of Norfloxacin in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:
In vitro, quantitative determination of Norfloxacin
Sensitivity: 0.03 ppb (ng/mL)
Detection Range: Tissue (chicken, porcine, fish, shrimp) - 0.06 ppb, Honey - 0.08 ppb, Milk - 0.6 ppb, Milk powder - 2 ppb, Eggs - 0.9 ppb, Urine - 0.15 ppb
Sample recovery rate: Tissue, Honey, Milk, Milk powder, Eggs - 85%±15%
Cross-reactivity: Norfloxacin - 100%

III. Sample Type:
Tissue, Urine, Feed

IV. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>E4776-100</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro ELISA Plate</td>
<td>96 wells</td>
<td>E4776-100-1</td>
</tr>
<tr>
<td>Standard</td>
<td>6 X 1 ml</td>
<td>E4776-100-2</td>
</tr>
<tr>
<td>HRP Conjugate</td>
<td>5.5 ml</td>
<td>E4776-100-3</td>
</tr>
<tr>
<td>Antibody Working Solution</td>
<td>5.5 ml</td>
<td>E4776-100-4</td>
</tr>
<tr>
<td>Substrate Reagent A</td>
<td>6 ml</td>
<td>E4776-100-5</td>
</tr>
<tr>
<td>Substrate Reagent B</td>
<td>6 ml</td>
<td>E4776-100-6</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>6 ml</td>
<td>E4776-100-7</td>
</tr>
<tr>
<td>Wash Buffer (20X)</td>
<td>40 ml</td>
<td>E4776-100-8</td>
</tr>
<tr>
<td>Reconstitution Buffer (5X)</td>
<td>50 ml</td>
<td>E4776-100-9</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td>3</td>
<td>E4776-100-10</td>
</tr>
</tbody>
</table>

V. User Supplied Reagents and Equipment:
- Microplate reader capable of measuring absorbance at 450 nm
- 0.15 M HCl
- Clean Eppendorf tubes for preparing standards or sample dilutions

VI. Storage and Handling:
Store at 4°C.

VII. Reagent and Sample Preparation:
Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Wash Buffer (20X):** Dilute 20X Concentrated Wash Buffer to 1X with deionized water.
- **Reconstitution Buffer (5X):** Dilute 5X Reconstitution Buffer with deionized water. Mix 5x Reconstitution Buffer (V): Deionized water (V) = 1:4. The Reconstitution buffer can be store at 4°C for a month.
- **0.15 M HCl:** Dissolve 5 ml of Concentrated hydrochloric acid (HCl) to 400 ml.
- **Standard:**

<table>
<thead>
<tr>
<th>Concentration (ppb)</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>0</td>
<td>0.03</td>
<td>0.09</td>
<td>0.27</td>
<td>0.81</td>
<td>2.43</td>
</tr>
</tbody>
</table>
VIII. Sample Preparation:

Sample pretreatment:

- **Pretreatment of tissue:**
  Weigh 2 ± 0.05 g of crushed homogenate tissue sample; add 8 mL of 0.1 M NaOH Solution. Mix well for 5 min, centrifuge at a speed of over 4000 r/min for 10 min at room temperature. Remove 2 ml of the clear upper organic layer solution to a clean and dry glass tube, dry at 50-60°C with nitrogen evaporators or water bath. Add 1 ml of N hexane and shake for 2 min. Add 1 ml of Reconstitution Buffer Solution and shake for 30 sec to mix well. Centrifuge for 5 min at 4000 r/min at room temperature. Remove the N hexane upper layer, take 50 μl of the lower water layer solution for analysis.
  
  **Note:** Sample dilution factor: 2, minimum detection limit: 0.06 ppb.

- **Pretreatment of honey sample:**
  Weigh 1 ± 0.05 g of honey into a 50 ml tube, add 6 mL of Sample Extraction Solution and shake for 5 min to ensure it is thoroughly dissolved. Add 3 ml of Reconstitution Buffer and 11 ml of Dichloromethane, shake well for 5 min. Then centrifuge at 4000 r/min for 5 min at room temperature. Remove the supernatant and transfer 8 ml of the upper layer organic solution to a dry container. Dry at 50-60 °C with nitrogen evaporators or water bath. Dissolve the dry residue with 1 ml of Reconstitution Buffer. Add 1 ml of N hexane and oscillate for 30 s. Centrifuge for 5 min at 3 000 r/min at room temperature. Remove the N hexane upper layer take 50 μl of the lower layer solution for analysis.
  
  **Note:** Sample dilution factor: 2, minimum detection limit: 0.08 ppb.

- **Pretreatment of milk sample:**
  Dilute the milk with Reconstitution Buffer for 20 times (eg add 25 μl of milk into 475 μl of Reconstitution Buffer), shake for 1 min to dissolve it well. Take 50 μl for detection and analysis.
  
  **Note:** Sample dilution factor: 20, minimum detection limit: 0.6 ppb

- **Pretreatment of milk powder sample:**
  Weigh 0.5 ± 0.02 g of homogenate sample into a 10 ml tube, add 5 mL of deionized water and oscillate to dissolve well. Mix 100 μl of sample solution with 400 μl of Reconstitution Buffer. Dissolve for 1 min. Take 50 μL for detection and analysis.
  
  **Note:** Sample dilution factor: 50, minimum detection limit: 2 ppb

- **Pretreatment of eggs sample:**
  Weigh 1 ± 0.02 g of homogenate egg into a 10 ml tube, add 5 ml of deionized water and shake to dissolve it well. Mix 100 μl of sample solution with 400 μl of Reconstitution Buffer. Shake well for 1 min. Take 50 μl for detection and analysis.
  
  **Note:** Sample dilution factor: 30 minimum detection limit: 0.9 ppb

- **Pretreatment of urine sample:**
  Add 4 ml of Reconstitution Buffer Solution 3 into 1 mL of clear urine sample, mix for 30 s. Take 50 μl for detection and analysis.
  
  **Note:** Sample dilution factor: 5, minimum detection limit: 0.15 ppb

IX. 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 50 μl of each standard or samples into appropriate wells.
  2. Add 50 μl of HRP Conjugate to each well. Add 50 μl of Antibody Working Solution. Cover the plate with the sealer provided in the kit. Gently mix and incubate for 45 min. at 25°C.
  3. Aspirate the solution from each well add 300 μl of 1x wash buffer to each well. Leave it for 30 sec, aspirate the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 5 times.
  4. Repeat wash Step 3.
  5. Add 50 μl of Substrate Reagent A to each well and then add 50 μl of Substrate Reagent B. Cover with a plate sealer. Incubate for about 15 min at 25°C. Protect the plate from light.
  6. Add 50 μl of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
  7. Read the absorbance in micro plate reader set to 450 nm reference wavelength 630 nm. This step should be performed within 10 min after stop reaction.

X. Calculation:

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.
Absorbance (%) = \( \frac{A}{A_0} \times 100\% \)

\( A \): Average absorbance of standard or sample

\( A_0 \): Average absorbance of 0 ppb Standard

Typical standard curve and data is provided below for reference only. A standard curve must be run with each assay.

<table>
<thead>
<tr>
<th>Concentration of standard (ppb)</th>
<th>OD-1</th>
<th>OD-2</th>
<th>Average OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.1148</td>
<td>2.2127</td>
<td>2.1638</td>
</tr>
<tr>
<td>0.03</td>
<td>1.6810</td>
<td>1.7729</td>
<td>1.7270</td>
</tr>
<tr>
<td>0.09</td>
<td>1.3458</td>
<td>1.3795</td>
<td>1.3624</td>
</tr>
<tr>
<td>0.27</td>
<td>0.9256</td>
<td>0.9121</td>
<td>0.9189</td>
</tr>
<tr>
<td>0.81</td>
<td>0.4668</td>
<td>0.4880</td>
<td>0.4774</td>
</tr>
<tr>
<td>2.43</td>
<td>0.2324</td>
<td>0.2335</td>
<td>0.2330</td>
</tr>
</tbody>
</table>

XI. RELATED PRODUCTS:

- Vancomycin ELISA Kit (E4605)
- Gentamicin (serum/urine) ELISA Kit (K4315)
- Diazepam ELISA Kit (E4772)
- Enrofloxacin (ENR) ELISA Kit (E4277)

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