Ciprofloxacin (Cipro) ELISA Kit

(Catalog # E4365-100, 100 assays, Store at 4°C)

I. Introduction:
Ciprofloxacin is an antibiotic used to treat a number of bacterial infections. It is commonly used to treat acne and rosacea. Historically it was important in reducing the number of deaths from cholera. A broad-spectrum antibiotic of the polyketide class, it is produced by the actinobacterial genus Streptomyces. It acts by inhibiting protein synthesis important in reducing the number of deaths from cholera. BioVision's Ciprofloxacin ELISA kit is a competitive ELISA assay for the quantitative measurement of Ciprofloxacin in tissues, honey and urine. The density of color is proportional to the amount of tetracycline captured from the samples.

II. Application:
This ELISA kit is used for in vitro quantitative determination of Ciprofloxacin.
Detection Range: 0.5 – 40 ppb (ng/ml)
Sensitivity: < 0.05 ppb
Detection limit: 0.3 ppb for tissue, 0.4 ppb for honey, 3 ppb for milk and egg, 6 ppb for milk powder
Cross Reactivity: Sarafloxacin – 110%; Oxolin acid – 28%; Levofloxacin – 10%; Lomefloxacin, Marbofloxacin – 4%

III. Sample Type:
Tissue, honey, eggs, milk, milk powder

IV. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>E4365-100</th>
<th>Part No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro ELISA Plate</td>
<td>8 X 12 strips</td>
<td>E4365-100-1</td>
</tr>
<tr>
<td>Standard (S1 – S6)</td>
<td>1.0 ml X 6</td>
<td>E4365-100-2-x</td>
</tr>
<tr>
<td>High standard (100 ppb)</td>
<td>1.0 ml</td>
<td>E4365-100-3</td>
</tr>
<tr>
<td>Antibody working solution</td>
<td>5.5 ml</td>
<td>E4365-100-4</td>
</tr>
<tr>
<td>Enzyme conjugate</td>
<td>5.5 ml</td>
<td>E4365-100-5</td>
</tr>
<tr>
<td>Substrate A solution</td>
<td>6 ml</td>
<td>E4365-100-6</td>
</tr>
<tr>
<td>Substrate B solution</td>
<td>6 ml</td>
<td>E4365-100-7</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>5 ml</td>
<td>E4365-100-8</td>
</tr>
<tr>
<td>Concentrated Wash Solution (20X)</td>
<td>40 ml</td>
<td>E4365-100-9</td>
</tr>
<tr>
<td>Concentrated Redissolving solution (5X)</td>
<td>50 ml</td>
<td>E4365-100-10</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td>1</td>
<td>E4365-100-11</td>
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</tbody>
</table>

V. User Supplied Reagents and Equipment:
- Reagents: 0.15M HCl, anhydrous acetonitrile, N-hexane, dichloromethane (CH₂Cl₂)
- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes 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.

VII. Reagent Preparation:
Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. Standards: Dilute each standard 10 times with diluted redissolving solution as the working solution "right before" the experiment.

<table>
<thead>
<tr>
<th>Tube #</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td></td>
<td>0</td>
<td>0.1</td>
<td>0.3</td>
<td>0.9</td>
<td>2.7</td>
<td>8.1</td>
</tr>
</tbody>
</table>

2. Redissolving solution: Dilute the concentrated redissolving solution 5 times with deionized water to be used for sample redissolving, it can be stored at 4°C environment up to a month.
3. Wash Buffer: Dilute 40 ml of the concentrated washing buffer with the distilled or deionized water to 800 ml (or just to the required volume) for using.
4. Sample Extracting Solution: Mix 10ml 0.15M HCl with 90 ml anhydrous acetonitrile, mix completely.
5. Sample Preparation:
Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.

FOR RESEARCH USE ONLY! Not to be used on humans.
**Tissue, liver, egg samples**: Weigh 2 g homogeneous sample into 50 ml centrifuge tube, Add 8 ml sample extract solution, mix with vortex for 5 min, centrifuge at 4000 r/min at room temperature for 10 min. Take 2 ml clear organic phase of upper into the 10 ml glass tube, and dry at 50 to 60°C with nitrogen or water bath. Add 1 ml N-hexane, mix with vortex for 2 min, then add 1 ml redissolving solution, mix with vortex for 30 seconds, centrifuge at 4000 r/min at room temperature for 5 min. Wipe out the upper N-hexane; take 50 μl Lower water phase to be analyses. (Dilution factor: 2)

**Honey**: Weigh 1.0 g homogeneous sample into 50 ml centrifuge tube, add 6 ml sample extract solution, oscillate 5 min to make it dissolve completely. Add 3 ml redissolving solution, then add 11 ml dichloromethane (CH₂Cl₂), oscillate 5 min, centrifuge at 4000 r/min at room temperature for 5 min. Wipe out the upper phase, take 8 ml organic phase to dry container, and dry at 50 to 60°C with nitrogen or water bath. Dissolve the dry residue with 1 ml redissolving solution, then add 1 ml N-hexane, mix 30 s, centrifuge at 3000 r/min at room temperature for 5 min. Wipe out the upper phase; take 50 μl Lower phase to be analyses. (Dilution factor: 2)

**Milk Sample**: Take 25 μl milk sample and 475 μl redissolving solution, mix and oscillate 1 min. Use 50 μl solution to be analyses. (Dilution factor: 20)

**Milk Powder**: Weigh 0.5 g homogeneous sample into 10 ml centrifuge tube, add 5 ml deionized water, oscillate and make it dissolve fully. Take 100 μl sample and 400 μl redissolving solution (Liquor 3), mix and oscillate 1 min. Use 50 μl solution to be analyses. (Dilution factor: 50)

**Egg sample**: Weigh 1.0 g homogeneous sample into 10 ml centrifuge tube, add 5 ml deionized water, oscillate and make it dissolve fully. Take 100 μl sample and 400 μl redissolving solution, mix and oscillate 1 min. Use 50 μl solution for analysis. (Dilution factor: 30)

### VIII. Assay Protocol:

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

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 50 μl diluted standards or samples into marked well. Add 50 μl Enzyme conjugate and 50 μl antibody working solution into each well.
3. Oscillate the plate for 5 sec, cover the well and incubate in dark for 45 min at RT (25°C).
4. Discard solution, wash plate 5 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (250 μl) using a multi-channel pipette or autowasher. Let it soak for 1 min, and then remove all residual wash-liquid from the wells. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
5. Add 50 μl Substrate A solution, then add 50 μl Substrate B solution to each well, oscillate gently for 5 s, incubate for 15 min at RT in dark.
6. Add 50 μl Stop Solution to each well and oscillate gently to stop the reaction.
7. Read result at 450 nm within 10 minutes.

### IX. CALCULATION:

Percentage of absorbance value (%) = A/A₀ X 100%

A: the average (double wells) OD value of the sample or the standard solution; A₀: the average OD value of the 0 ppb standard solution.

To draw the standard curve and calculate, take absorbance percentage of standards as Y-axis, the corresponding log of standards concentration (ppb) as X-axis. Draw the standard semilog curves with X-axis and Y-axis. Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve; last, Multiply by the corresponding dilution times is the actual concentration of Sal of samples.

### X. RELATED PRODUCTS:

- Salbutamol (SALB) ELISA Kit (Cat. No. K4209-100)
- Sulfonamides residue ELISA Kit (Cat. No. K4207-100)
- Aflatoxin B1 (AFB1) ELISA Kit (Cat. No. K4208-100)
- Fluoroquinolones ELISA Kit (Cat. No. K4205-100)
- Gentamicin ELISA Kit (Cat. No. K4206-100)
- Gentamicin (serum/urine) ELISA Kit (Cat. K4315-100)
- Kanamycin ELISA Kit (Cat. No. K4210-100)
- Streptomycin ELISA Kit (Cat. No. E4272-100)
- Melamine ELISA Kit (Cat. No. E4274-100)