Sulfametoxydiazine ELISA Kit
(Catalog # E4775-100; 96 assays, Storage at 4°C)

I. Introduction:
Sulfametoxydiazine is a long-acting sulfonamide antibacterial. It is used as a leprostatic agent and in the treatment of urinary tract infections. BioVision Sulfametoxydiazine ELISA Kit is based on the Competitive ELISA principle. It can detect Sulfametoxydiazine in tissue, serum, honey, milk, urine samples. The micro-plate provided in this kit has been pre-coated with Sulfametoxydiazine. During the reaction, Sulfametoxydiazine in the samples or standard competes with Sulfametoxydiazine coated on the plate for binding to the anti-Sulfametoxydiazine 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 Sulfametoxydiazine. The concentration of Sulfametoxydiazine in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:
In vitro, quantitative determination of Sulfametoxydiazine
Sensitivity: 0.05 ppb (ng/mL)
Detection Range: Tissue (high detection limit) - 0.5 ppb, Tissue (low detection limit) - 0.25 ppb, Serum/urine - 0.2 ppb, Honey- 0.05 ppb, Milk- 1 ppb.
Sample recovery rate: Tissue/honey - 95% ± 25%, Urine/milk/serum/ feed - 85% ± 25%
Cross-reactivity: Sulfadiazine 40%, Sulfamerazine 25%, Sulfadoxine 35%

III. Sample Type:
Tissue, Serum, Urine, Honey, Milk

IV. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>E4775-100</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro ELISA Plate</td>
<td>96 wells</td>
<td>E4775-100-1</td>
</tr>
<tr>
<td>Standard</td>
<td>6 X 1 ml</td>
<td>E4775-100-2</td>
</tr>
<tr>
<td>HRP Conjugate</td>
<td>5.5 ml</td>
<td>E4775-100-3</td>
</tr>
<tr>
<td>Antibody Working Solution</td>
<td>5.5 ml</td>
<td>E4775-100-4</td>
</tr>
<tr>
<td>Substrate Reagent A</td>
<td>6 ml</td>
<td>E4775-100-5</td>
</tr>
<tr>
<td>Substrate Reagent B</td>
<td>6 ml</td>
<td>E4775-100-6</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>6 ml</td>
<td>E4775-100-7</td>
</tr>
<tr>
<td>Wash Buffer (20X)</td>
<td>40 ml</td>
<td>E4775-100-8</td>
</tr>
<tr>
<td>Reconstitution Buffer (2X)</td>
<td>50 ml</td>
<td>E4775-100-9</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td>3</td>
<td>E4775-100-10</td>
</tr>
</tbody>
</table>

V. User Supplied Reagents and Equipment:
- Microplate reader capable of measuring absorbance at 450 nm
- 0.2 M NaOH, 0.02 M PB Buffer, 0.5 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 (2X):** Dilute 2X Reconstitution Buffer with deionized water. Mix 10x Reconstitution Buffer (V): Deionized water (V) =1:1). The Reconstitution buffer can be store at 4°C for a month.
- **0.2 M NaOH :** Dissolve 0.8 g NaOH with 100 ml deionized water
- **0.02 M PB Buffer:** Dissolve 2.58 g of Na₂HPO₄·12H₂O and 0.44 g of NaH₄PO₄·2H₂O to 500 mL of deionized water
- **0.5 M HCl:** Add 4.3 ml of concentrated HCl into 100 mL of deionized water and mix thoroughly
VIII. Sample Preparation:

Sample pretreatment:

- **Pretreatment of tissue (high detection limit):**
  Add 3 ± 0.05 g of homogeneous tissue sample to a centrifuge tube, and then add 3 ml of 0.02M PB buffer shake and mix thoroughly. Add 4 ml of ethyl acetate and 2 ml of acetonitrile, mix for 10 min, centrifuge at above 4000 r/min for 10 min. Take 2 ml of upper liquid (about 1 g of sample), blow-dry at 50-60 °C with nitrogen Evaporators/Water bath. Dissolve the residual with 1 ml of n-hexane, add 1 mL of Reconstitution buffer and shake for 1 min. Centrifuge at 4000 r/min for 5 min. Discard the upper n-hexane, take 50 μl of lower liquid for analysis.
  **Note:** Sample dilution factor: 1, minimum detection dose: 0.05 ppb

- **Pretreatment of tissue (low detection limit):**
  Add 2±0.05 g of homogeneous tissue sample to a centrifuge tube, and then add 8 ml of 0.02 M PB buffer shake for 2 min, centrifuge at above 4000 r/min for 10 min. Take 50 μl of liquid for analysis.
  **Note:** Sample dilution factor: 5, minimum detection dose: 0.25 ppb

- **Pretreatment of serum:**
  Put blood sample at room temperature for 30 min, centrifuge at above 4000 r/min for 10 min, separate the serum or filter the serum. Take 1 ml of serum, add 3 ml of 0.02 M PB buffer, and mix for 30 sec. Take 50 μl of liquid for analysis.
  **Note:** Sample dilution factor: 4, minimum detection dose: 0.2 ppb

- **Pretreatment of honey:**
  Weigh 1±0.05 g of honey sample into 50 ml a centrifuge tube, and then add 1 ml of 0.5 M HCl solution, put at 37°C for 30 min. Add 2.5 ml of 0.2 M NaOH solution (adjust the PH value to about 5), then add 4 ml of ethyl acetate, shake for 5 min, centrifuge at above 4000 r/min at room temperature for 10 min. Take 2 mL of upper liquid, blow-dry at 50-60°C with nitrogen or air. Add 0.5 ml of Reconstitution buffer and mix for 30 sec. Take 50 μl of liquid for analysis.
  **Note:** Sample dilution factor: 1, minimum detection dose: 0.05 ppb

- **Pretreatment of urine:**
  Mix 3 ml of 0.02 M PB buffer solution and 1 ml of centrifuged clear urine sample for 30 sec. Take 50 μl of liquid to analysis.
  **Note:** Sample dilution factor: 4, minimum detection dose: 0.2 ppb

- **Pretreatment of milk:**
  Dilute milk sample with 0.02 M PB buffer solution with the ratio of 1:20 (for example, 20 μl milk + 380 μL of 0.02 M PB buffer), mix for 30 sec. Take 50 μl of liquid to analysis.
  **Note:** Sample dilution factor: 20, minimum detection dose: 1 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** and 50 μl of **Antibody Working Solution** to each well. 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. Add 50 μl of **Substrate Reagent A** to each well and then add 50 μl of **Substrate Reagent B**. Cover with a new plate sealer. Incubate for about 15 min at 25°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
5. 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.
6. Read the absorbance in micro plate reader set to 450 nm reference wavelength 630 nm. This step should be performed within 5 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>2.7933</td>
<td>2.6526</td>
<td>2.7230</td>
</tr>
<tr>
<td>0.05</td>
<td>2.1833</td>
<td>2.2208</td>
<td>2.2021</td>
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<tr>
<td>0.15</td>
<td>1.6770</td>
<td>1.7502</td>
<td>1.7136</td>
</tr>
<tr>
<td>0.45</td>
<td>1.0385</td>
<td>1.1043</td>
<td>1.0714</td>
</tr>
<tr>
<td>1.55</td>
<td>0.4833</td>
<td>0.3073</td>
<td>0.4953</td>
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<tr>
<td>4.05</td>
<td>0.1248</td>
<td>0.1327</td>
<td>0.1288</td>
</tr>
</tbody>
</table>

XI. RELATED PRODUCTS:

- Sulfadiazine ELISA Kit (E4773)
- Diazepam ELISA Kit (E4772)
- Enrofloxacin (ENR) ELISA Kit (E4277)
- Sulfamonomethoxine ELISA Kit (E4774)

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