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Sulfamonomethoxine ELISA Kit

10/19

(Catalog # E4774-100; 96 assays, Storage at 4°C)

I. Introduction:

Sulfamonomethoxine is a long-acting sulfonamide antibacterial agent, used in blood kinetic studies, and blocks the synthesis of folic acid by inhibiting synthetase of dihydropteroate. BioVision Sulfamonomethoxine ELISA Kit is based on the Competitive ELISA principle. It can detect Sulfamonomethoxine in tissue, serum, honey, milk, urine samples. The micro-plate provided in this kit has been pre-coated with Sulfamonomethoxine. During the reaction, Sulfamonomethoxine in the samples or standard competes with Sulfamonomethoxine coated on the plate for binding to the anti-Sulfamonomethoxine antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microplate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of Sulfamonomethoxine. The concentration of Sulfamonomethoxine in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

In vitro, quantitative determination of Sulfamonomethoxine
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%, Sulfametoxydiazine 50%, Sulfadoxine 35%

III. Sample Type:

Tissue, Serum, Urine, Honey, Milk

IV. Kit Contents:

Components	E4774-100	Part Number
Micro ELISA Plate	96 wells	E4774-100-1
Standard	6 X 1 ml	E4774-100-2
HRP Conjugate	5.5 ml	E4774-100-3
Antibody Working Solution	5.5 ml	E4774-100-4
Substrate Reagent A	6 ml	E4774-100-5
Substrate Reagent B	6 ml	E4774-100-6
Stop Solution	6 ml	E4774-100-7
Wash Buffer (20X)	40 ml	E4774-100-8
Reconstitution Buffer (2X)	50 ml	E4774-100-9
Plate Sealer	3	E4774-100-10

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 4ºC**.**

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℃ 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 $Na_2HPO4 \cdot 12H_2O$ and 0.44 g of $NaH_2PO4 \cdot 2H_2O$ 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

- **Standard:**

Standard	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
Concentration (ppb)	0	0.05	0.15	0.45	1.35	4.05

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 ℃ 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±0.05 g of homogeneous tissue sample to a centrifuge tube, 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: 2 ppb

- **Pretreatment of honey:**
  Weigh 1± 0.05 g of honey sample into 50 ml a centrifuge tube, add 1 ml of 0.5 M HCl solution, put at 37℃ 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℃ 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: 0.5 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: 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: 10 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.

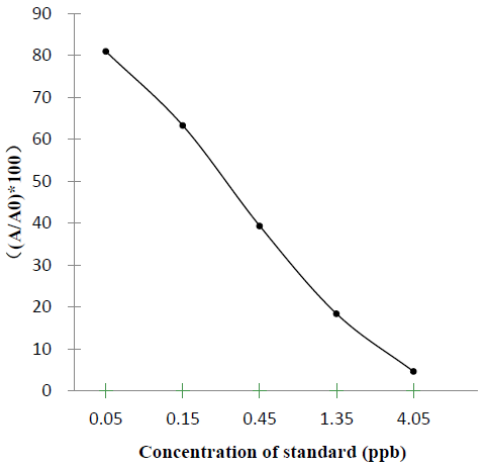
X. Calculation:

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 (%)=A/A_0 ×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

Concentration of standard (ppb)	OD-1	OD-2	Average OD
0	2.7843	2.6624	2.7234
0.05	2.1945	2.2124	2.2035
0.15	1.6898	1.7541	1.7220
0.45	1.0354	1.1042	1.0698
1.35	0.4933	0.5045	0.4989
4.05	0.1247	0.1241	0.1244

XI. RELATED PRODUCTS:

- Sulfaquinoxaline ELISA Kit (E4773)
- Enrofloxacin (ENR) ELISA Kit (E4277)
- Chloramphenicol (CAP) ELISA Kit (K4230)
- Ciprofloxacin (Cipro) ELISA Kit (E4365)

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