Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Sulfamethazine ELISA Kit

11/19

(Catalog # E4778-100; 96 assays, Storage at 4°C)

I. Introduction:

Sulfamethazine is a sulfonamide consisting of pyrimidine with methyl substituents at the 4- and 6-positions and a 4-aminobenzenesulfonamido group at the 2-position. It has a role as an anti-infective agent, a carcinogenic agent, a ligand, an antibacterial drug, an antimicrobial agent. Sulfamethazine ELISA Kit is based on the Competitive ELISA principle. The micro-plate provided in this kit has been pre-coated with Sulfamethazine. During the reaction, Sulfamethazine in the samples or standard competes with Sulfamethazine coated on the plate for binding to the anti-Sulfamethazine 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 Sulfamethazine. The concentration of Sulfamethazine in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

In vitro, quantitative determination of Sulfamethazine
Sensitivity: 0.5 ppb (ng/mL)
Detection Range: Tissue (high detection limit method) - 0.5 ppb, Tissue (low detection limit method) - 2.5 ppb, Serum, Urine - 2 ppb, Honey- 0.5 ppb, Milk -10 ppb

Sample recovery rate: 95%±25%, Urine, Milk, Serum- 85%±25%.

Cross-reactivity: Sulfamethazine - 100%

III. Sample Type:

Tissue, Urine, Feed

IV. Kit Contents:

Components	E4778-100	Part Number
Micro ELISA Plate	96 wells	E4778-100-1
Standard	6 X 1 ml	E4778-100-2
HRP Conjugate	5.5 ml	E4778-100-3
Antibody Working Solution	5.5 ml	E4778-100-4
Substrate Reagent A	6 ml	E4778-100-5
Substrate Reagent B	6 ml	E4778-100-6
Stop Solution	6 ml	E4778-100-7
Wash Buffer (20X)	40 ml	E4778-100-8
Reconstitution Buffer (2X)	50 ml	E4778-100-9
Plate Sealer	3	E4778-100-10

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- anhydrous acetonitrile、n-hexane、concentrated HCl, methylene dichloride
- 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 2x Reconstitution Buffer (V): Deionized water (V) =1:1). The Reconstitution buffer can be store at 4℃ for a month.
- **0.5 M HCl:** Dissolve 4.3 ml of concentrated hydrochloric acid (HCl) to 100 ml.
- **0.2 M NaOH Solution:** Dissolve 0.8 g of NaOH to 100 mL deionized water.
- **0.02 M PB Buffer:** Dissolve 2.58 g of $Na_2HPO_412H_2O$ and 0.44 g of $NaH_2PO_42H_2O$ to 500 mL deionized water
- **Standard:**

Standard	S1	S2	S3	S4	S5	S6
Concentration (ppb)	0	0.5	1.5	4.5	13.5	40.5

VIII. Sample Preparation:

Sample pretreatment:

- **Pretreatment of tissue (high detection limit):**
  Weigh 3 ± 0.05 g of homogenate tissue sample; add 3 mL of 0.02 M PB Buffer, oscillate until it mixed fully. Then add 4 mL of Ethyl acetate and 2 mL of Acetonitrile, shake well for 10 min, centrifuge at 4000 r/min for 10 min at room temperature. Take 2 mL of the upper layer liquid and dry at 50-60℃ with nitrogen evaporators/water bath. Add 1 mL of N-hexane to dissolve the remaining dry material, then add 1 mL of Reconstitution Buffer. Mix well for 1 min and centrifuge at 4000 r/min for 5 min. Remove the upper layer N-hexane phase, take 50 µl of the lower layer liquid for analysis.
  Note: Sample dilution factor: 1, minimum detection limit: 0.5 ppb.

- **Pretreatment of tissue (low detection limit):**
  Weigh 2 ± 0.05 g of homogenate tissue sample into a centrifuge tube, add 8 ml of 0.02 M PB Buffer, shake well for 2 min. Centrifuge at 4000 r/min for 10 min. Take 50 µl for analysis.
  Note: Sample dilution factor: 5, minimum detection limit: 2.5 ppb.

- **Pretreatment of honey:**
  Weigh 1 ± 0.05 g of honey sample into a 50 ml centrifuge tube, add 1 ml of 0.5 M HCl Solution, incubate for 30 min at 37℃. Add 2.5 mL of 0.2 M NaOH solution (the pH should be adjusted to about 5), then add 4 mL of Ethyl acetate and oscillate for 5 min. Centrifuge at 4000 r/min for 10 min at room temperature. Take 2 mL of the upper layer liquid and dry at 50-60°C with nitrogen evaporators/water bath. Add 0.5 mL of Reconstitution Buffer, mix for 30s. Take 50 µl for analysis.
  Note: Sample dilution factor: 1, minimum detection limit: 0.5 ppb

- **Pretreatment of swine urine sample:**
  Mix 3 mL of 0.02 M PB Buffer and 1 ml of centrifuged clear urine sample for 30s. Take 50 µl for analysis.
  Note: Sample dilution factor: 4, minimum detection limit: 2 ppb

- **Pretreatment of milk sample:**
  Dilute the milk sample with 0.02 M PB Buffer for 20 times (e.g.: 20 µl +380 µl of 0.02 M PB Buffer), mix for 30s. Take 50 µl for analysis.
  Note: Sample dilution factor: 20 minimum detection limit: 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** 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. 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.
   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 10 min after stop reaction.

X. Calculation:

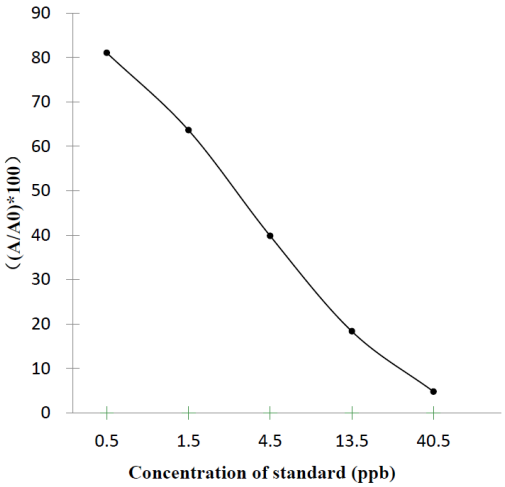
Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

Absorbance (%)=A/A_0 ×100%

A: Average absorbance of standard or sample

A_0 : Average absorbance of 0 ppb Standard

Concentration of standard (ppb)	OD-1	OD-2	Average OD
0	2.7543	2.6456	2.7000
0.5	2.1743	2.2028	2.1886
1.5	1.6870	1.7502	1.7186
4.5	1.0375	1.1143	1.0759
13.5	0.4833	0.5083	0.4958
40.5	0.1284	0.1307	0.1296



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

XI. RELATED PRODUCTS:

- Sulfaquinoxaline ELISA Kit (E4773)
- Norfloxacin ELISA Kit (E4776)
- Sarafloxacin ELISA Kit (E4777)
- Sulfametoxydiazine ELISA Kit (E4775)
- Sulfamonomethoxine ELISA Kit (E4774)

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