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

Sulfamethoxazole ELISA Kit

10/19

(Catalog # E4770-100; 96 assays, Storage at 4°C)

I. Introduction:

Sulfamethoxazole is an antibiotic that blocks the synthesis of dihydrofolic acid by inhibiting the enzyme dihydropteroate synthase. It inhibits folic acid synthesis in prokaryotes. BioVision's Sulfamethoxazole ELISA Kit is based on the Competitive ELISA method. It can detect Sulfamethoxazole in samples, such as Tissue, Serum, Urine, Egg, Honey, Milk, Feed. The microtiter plate provided in the kit has been pre-coated with Sulfamethoxazole. During the reaction, Sulfamethoxazole in the samples or standard competes with coated Sulfamethoxazole on the solid phase supporter for sites of anti- Sulfamethoxazole antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and TMB substrate is added for color development. There is a negative correlation between the OD value of samples and the concentration of Sulfamethoxazole. The concentration of Sulfamethoxazole in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

In vitro, quantitative determination of Sulfamethoxazole
Detection Range: Tissue (high detection limit method) - 0.1 ppb, Tissue (low detection limit method) - 1 ppb, Honey- 0.1 ppb, Serum, Urine, Egg- 0.4 ppb, Milk- 2 ppb, Feed- 4 ppb,
Sensitivity: 0.1 ppb (ng/mL)

Sample recovery rate: Tissue/Honey/Egg ---85%±25%, Serum/Urine/Milk/Feed---80%±25%

III. Sample Type:

Tissue, Serum, Urine, Egg, Honey, Milk, Feed

IV. Kit Contents:

Components	E4770-100	Part Number
Micro ELISA Plate	96 wells	E4770-100-1
Standard	6 X 1 ml	E4770-100-2
HRP Conjugate	5.5 ml	E4770-100-3
Antibody Working Solution	5.5 ml	E4770-100-4
Substrate Reagent A	6 ml	E4770-100-5
Substrate Reagent B	6 ml	E4770-100-6
Stop Solution	6 ml	E4770-100-7
Wash Buffer (20X)	40 ml	E4770-100-8
Reconstitution Buffer (2X)	50 ml	E4770-100-9
Plate Sealer	3	E4770-100-10

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Methanol
- 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.
- **Standard:**

Standard	S1	S2	S3	S4	S5	S6
Concentration (ppb)	0	0.1	0.3	0.9	2.7	8.1

Solution preparation

- **0.1 M PBS Buffer :** Dissolve 25.8 g of $Na_2HPO4{\cdot}12H_2O$and 4.4g of $NaH2PO4{\cdot}2H_2O$ to1000 mL with deionized water
- **Acetonitrile-ethyl acetate**: Add 50 mL of Acetonitrile and 50 mL of Ethyl acetate to 100 mL glass bottle mix well.

- **0.5 M HCl**: Add 4.3 mL of Concentrated HCl to 100 mL with deionized water, mix well.
- **0.2 M NaOH:** Dissolve 0.8 g of NaOH to 100 mL with deionized water, mix fully.

VIII. Sample Preparation:

Sample pretreatment:

- **Pretreatment of tissue (high detection limit method):**
  Weigh 2±0.05 g of homogenate sample into 50 mL tube. Add 1ml of 0.1 M PBS Buffer; mix the sample to form a paste. Add 7 ml of Acetonitrile-ethyl acetate Solution. Mix for 2 min, centrifuge at 4000rpm for 5 min at room temperature. Take 4 ml of the clean organic layer to a dry container, dry at 50-60℃of nitrogen evaporators or water bath. Redissolve the dry residual sediment with 1 ml of N-hexane. Add 1 ml of Reconstitution Buffer and mix for 30s. Centrifuge at 4000 rpm for 5 min at room temperature. Remove the upper layer, and take 50 µl of the lower layer for analysis.
  Note: Sample dilution factor: 1, minimum detection limit: 0.1ppb

- **Pretreatment of tissue (low detection limit):**
  Weigh1±0.05g of homogenate into a 50 mL EP tube, add 9mL of 0.1 M PBS Buffer and shake for 5 min, centrifuge at 4000rpm for 5 min at room temperature. Take 50 µl of the supernatant for analysis.
  Note: Sample dilution factor: 10, minimum detection limit: 1ppb

- **Pretreatment of eggs:**
  Use homogenizer to homogenize egg sample, to mix egg white and egg yolk. Weigh 2 ± 0.05 g of homogenate sample into 50 mL EP tube. Add 8 mL of Acetonitrile and shake well for 10 min. Centrifuge at 4000 rpm for 5 min at room temperature. Take 1 mL of the supernatant to 10 mL clean dry glass dry at 50-60°C of nitrogen evaporators or water bath. Redissolve the dry residual sediment with 1 mL of N hexane. Add 1 mL of Reconstitution B buffer and mix for 1 min. Centrifuge at 4000 rpm for 5 min at room temperature. Discard the upper layer solution. Take 50 µl of the lower later solution for analysis is.
  Note: Sample dilution factor: 4, minimum detection limit 0.4 ppb

- **Pretreatment of swine serum:**
  Stand the serum for 30 min at room temperature. Centrifuge at 4000r/min for 10 min at room temperature, after the serum separated out. Take 1 mL of serum sample .Add 3 mL of 0.1 M PBS Buffer and oscillate fully for 30s.Take 50 µl for analysis.
  Note: Sample dilution factor: 4, minimum detection limit: 0.4ppb8th Edition, revised in February, 2018

- **Pretreatment of honey:**
  Weigh1± 0.05 g of honey sample into a 50 ml tube. Add 1mLof 0.5 M HCl Solution. Incubate at 37℃ for 30 min. Add 2.5mLof 0.2 M NaOH Solution (pH~5), then add4 mL Ethyl acetate. Oscillate for 5 min, centrifuge at 4000 rpm for 5 min at room temperature. Take 2mL of the supernatant to a dry container, dry at50-60℃of nitrogen evaporators or water bath. Redissolve the dry residual sediment with 0.5mL of Reconstitution Buffer**.** Mix for 30s.Take 50µlfor analysis.
  Note: Sample dilution factor: 1, minimum detection limit: 0.1 ppb

- **Pretreatment ofswine urine:**
  Add 3mL of 0.1 M PBS Buffer into 1mL of centrifuged clear urine sample, oscillate for 30s. Take 50 µl for detection and analysis.
  Note: Sample dilution factor: 4, minimum detection limit: 0.4ppb

- **Pretreatment of milk:**
  Dilute 100µl of milk with 0.1 M PBS Buffer (1:19, v/v, 100µlof milk +1.9mL of 0.1M PBS Buffer). Mix for 30s. Take 50 µl for analysis.
  Note: Sample dilution factor: 20, minimum detection limit: 2ppb

- **Pretreatment of feed:**
  Weigh 2.0±0.05g of feed sample into 50 mL centrifuge tube, add 8mLof Acetonitrile, oscillate 5min, and centrifuge at a speed over 4000 rpm for 5 min at room temperature. Take 1 mL of the upper organic layer to 10 mL clean dry glass, dry at 50-60℃ of nitrogen evaporators or water bath. Add1 mL of N-hexane, Oscillate for 30s, then add 1mL of 0.1 M PBS Buffer, mix sample for 30s, transfer sample to 2 centrifuge tube, centrifuge at 4000 r/min for 5min at room temperature. Remove the upper organic layer, take 100µlof the lower water layer to 2mL EP tube, add 900 µl of 0.1 M PBS Buffer, oscillate sample for 1min, mix well. Take 50 µl of sample for analysis.
  Note: Sample dilution factor: 40, minimum detection limit: 4 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 and 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. **Note:** solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and bubble formation as much as possible.
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.

Note: a microplate washer can be used in this step and other wash steps.

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:

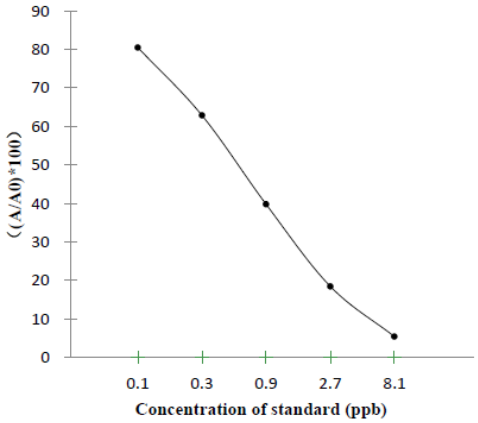
Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration 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	2.7943	2.6566	2.7255
0.1	2.1743	2.2118	2.1931
0.3	1.6747	1.7502	1.7125
0.9	1.0245	1.1413	1.0829
2.7	0.4873	0.5173	0.5023
8.1	0.1518	0.1417	0.1468

XI. RELATED PRODUCTS:

- Enrofloxacin (ENR) ELISA Kit (E4277)
- Chloramphenicol (CAP) ELISA Kit (K4230)
- Gentamicin ELISA Kit (K4206)
- Ciprofloxacin (Cipro) ELISA Kit (E4365)

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