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Sulfadiazine ELISA Kit

11/20

(Catalog # E4904-100; 96 assays, Store kit at -20 °C)

I. Introduction:

Sulfadiazine (SDZ) belongs to the class of broad-spectrum sulfonamide antibiotics and is widely used for the treatment of bacterial infections in poultry, cattle, beekeeping, humans etc. SDZ inhibits bacterial growth by inhibiting dihydropteroate synthetase that produces dihydropteroate, an important intermediate in folate synthesis. Additionally, SDZ can help prevent rheumatic fever, meningitis, chancroid, chlamydia, and infections by Haemophilus influenza. Sulfonamide residues may occur in food of animal origin such as meat, milk etc. and may cause serious side effects including nausea, loss of appetite, dizziness, rash etc. Therefore, sulfadiazine in some countries is allowed for use only in veterinary medicine. **BioVision's Sulfadiazine ELISA Kit** is a competitive enzyme immunoassay for the quantitative analysis of sulfadiazine concentration in a variety of sample types. In this assay, the sulfadiazine antigen is pre-coated on the 96-well plate. The SDZ in the sample and the antigen on the plate compete for binding to the anti-sulfadiazine antibody. After the addition of the enzyme conjugate that binds to captured SDZ antibody, TMB substrate is added and color signal is developed. The OD value of the sample is inversely proportional to the SDZ concentration in the sample. This value is compared to the sulfadiazine standard curve and the SDZ concentration is subsequently obtained. The kit is simple, easy and a rapid way to determine sulfadiazine concentration. The kit can detect 4 - 256 ng/ml SDZ concentration in less than 1 hr (LOD 2 ng/ml).

II. Application:

- *In vitro* quantitative determination of Sulfadiazine concentration in serum, urine, etc.

III. Sample Type:

- Serum and urine

IV. Species Reactivity:

- Universal

V. Kit Contents:

Components	E4904-100	Cap Code	Part Number
Pre-coated 96 Well Strip Plate	8 X 12 Strips	--	E4904-100-1
Sulfadiazine Standard	1 vial	Yellow	E4904-100-2
HRP Conjugate Stock	25 µl	Blue	E4904-100-3
Mouse Anti-Sulfadiazine Antibody	7 ml	NM	E4904-100-4
TMB Substrate	10 ml	Amber/NM	E4904-100-5
Stop Solution	10 ml	Blue/NM	E4904-100-6
Wash Buffer (10X)	50 ml	NM	E4904-100-7
Sample Diluent	20 ml	NM	E4904-100-8
Standard Buffer	20 ml	Red/NM	E4904-100-9
Conjugate Buffer	7.5 ml	Green/NM	E4904-100-10
Plate Sealers	4	--	E4904-100-11

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm and 650 nm
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended
- Eppendorf tubes
- Absorbent paper

VII. Storage and Handling:

Store kit at -20 °C for up to 12 months from the date of shipment. Opened kit is stable for 2 months at 4 °C.

VIII. Reagent Preparation:

Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. Bring all reagents to room temperature (RT) before use.

- **Sulfadiazine Standard:** Reconstitute the vial in 1.0 ml of Standard Buffer to prepare the **Sulfadiazine Standard Stock** solution. Allow the solution to sit at RT for 10 min. Vortex gently to mix. Store at -20 ºC.
- **HRP Conjugate Stock:** Spin briefly before opening the tube. Prepare the HRP Conjugate working solution before performing the assay.
- **Mouse Anti-Sulfadiazine Antibody, TMB Substrate, Stop Solution, Sample Diluent, Standard Buffer** and **Conjugate Buffer:** Ready to use. Store at 4 °C.
- **Wash Buffer (10X):** Bring bottle to RT. If crystals are present, warm up to RT and mix gently until the crystals are completely dissolved. Prepare 1X Wash Buffer for the assay. The 1X solution can be stored at 4 °C for one month.

IX. Sulfadiazine Standard Preparation:

1. Prepare 1 ml of **256 ng/ml Sulfadiazine Standard (S7)** by mixing 100 µl of **Sulfadiazine Standard Stock** solution with 900 µl of Standard Buffer.

2. Perform 2-fold serial dilutions of the Standards (as shown below) to prepare 0, 4, 8, 16, 32, 64, 128, and 256 ng/ml Sulfadiazine Standards. **Note:** Diluted Sulfadiazine Standards are stable for up to 3 weeks at -20 °C.

Standard	S7	S6	S5	S4	S3	S2	S1	S0
Standard Buffer		500 µl	500 µl	500 µl	500 µl	500 µl	500 µl	500 µl
	1 ml	500 µl S7	500 µl S6	500 µl S5	500 µl S4	500 µl S3	500 µl S2	--
	Mix well							
Final Standard Conc. (ng/ml)	256	128	64	32	16	8	4	0

X. Sample Preparation:

Urine sample

1. Centrifuge 0.2 ml of urine at 10,000 x g for 5 min to remove any precipitate. Transfer 150 µl of supernatant into a clean eppendorf tube.
2. Dilute the sample 10-fold with Sample Diluent (i.e, mix 20 µl Sample with 180 µl of Sample Diluent).
3. Use 50 µl per well for the assay.

Serum sample

1. Dilute serum 10-fold with Sample Diluent (i.e, mix 20 µl of serum with 180 µl of Sample Diluent).
2. Use 50 µl per well for the assay.

XI. Sulfadiazine ELISA Assay Protocol:

Notes:

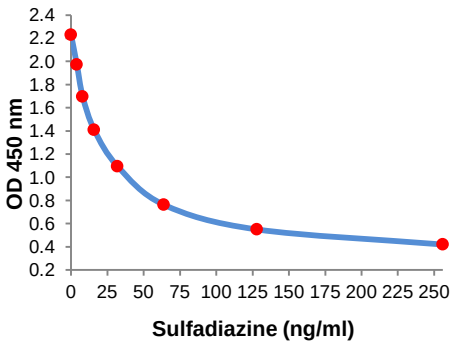
a. We recommended running all Standards and sample(s) in duplicates. A Standard Curve must be run with each assay
b. Prepare all reagents, Standards and sample(s) as instructed.
c. Prepare **HRP Conjugate working solution** by mixing 9 µl of HRP Conjugate Stock with 7.5 ml Conjugate Buffer and vortex for a min. The HRP Conjugate working solution is stable at 4 °C for 2 months.
d. Prepare 100 ml of **1X Wash Buffer** by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water.

1. Add 50 µl of **Standards or Sample(s)** into appropriate wells.
2. Add 50 µl of **HRP Conjugate working solution** and 50 µl of **Mouse Anti-Sulfadiazine Antibody** to the Standard and Sample wells.
3. Cover the microtiter plate with a plate sealer and mix well. Incubate the plate at RT (25 °C) for 50 min, protected from light.
4. Aspirate all the reagents and wash each well 5 times. Wash by filling each well with 250 µl of **1X Wash Buffer** and incubating for 30 sec. Remove the 1X Wash Buffer completely before the next wash. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials. **Note:** Complete removal of Wash Buffer is essential for accurate results.
5. Add 100 µl of **TMB Substrate** to each well. Tap or shake the plate occasionally to ensure complete mixing.
6. Measure the OD of the Sulfadiazine Standard well **(S0)** at 650 nm. When the reading is ~ 0.8, add 50 µl of **Stop Solution** and gently tap the plate to ensure thorough mixing.
7. Measure the OD of the plate at 450 nm at RT.

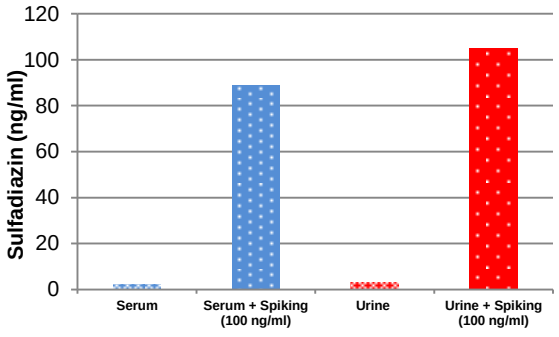
XII. Calculation:

Plot the Sulfadiazine Standard Curve as relative OD 450 nm of each Sulfadiazine Standard (Y) vs. the respective sulfadiazine concentrations (X). The concentration of sulfadiazine in sample(s) is calculated from the Sulfadiazine Standard Curve and reported as ng/ml. **Note:** If the Sample(s) are diluted, multiply the dilution factor to the concentrations from interpolation.

A.



B.



Figures. A. Sulfadiazine Standard Curve. *This Standard Curve is for demonstration only.* **B.** Spike recovery experiment using human serum and urine samples spiked with Sulfadiazine (100 ng/ml) respectively. Spike recovery was 85-100%.

XIII. Related Products:

Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315)
Folic Acid ELISA Kit (Cat. No. E4523)
Caffeine Acid ELISA Kit (Cat. No. E4558)
Isoniazid ELISA Kit (Cat. No. E4765)
Ampicillin ELISA Kit (Cat. No. E4350)
Quinolone ELISA Kit (Cat. No. E4530)
Vancomycin ELISA Kit (Cat. No. E4605)
Carnosine ELISA Kit (Cat. No. E4766)

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