Sulfamerazine (SM1) ELISA Kit

Catalog No.: abx365118

Size: 96 tests

Storage: Store at 4°C.

Application: The quantitative detection of Sulfamerazine in Milk, Honey, Eggs, and Muscle tissue.

Detection Limit: Milk -- 10 ng/ml; Honey -- 0.5 ng/ml; Egg -- 0.25 ng/ml; Muscle -- 0.5 ng/ml

Introduction: Sulfamerazine (SM1) is a member of the sulfonamides, a large class of antimicrobial drugs used widely in veterinary care. Sulfonamides slow the course of infections by inhibiting the enzyme dihydropteroate synthase, preventing pathogens from synthesizing vitamin B9, a molecule essential to bacterial growth. As Sulfamerazine cannot kill pathogens on its own, and as sulfonamide resistance is becoming increasingly common, Sulfamerazine is typically used in conjunction with other antibiotics as an initial treatment for skin infections, burns, and coccidiosis.

Principle of the Assay: This kit is based on competitive enzyme-linked immuno-sorbent assay technology. Sulfamerazine antigen is precoated onto a 96-well plate. The standards and samples and a biotin-conjugated antibody specific to Sulfamerazine are added to the wells and incubated. After washing away the unbound conjugates, avidin conjugated to Horseradish Peroxidase is added to each microplate well. After TMB substrate solution is added only wells that contain bound Sulfamerazine antibody will produce a blue color product that changes into yellow after adding the stop solution. The intensity of the yellow color is inversely proportional to the Sulfamerazine amount present in the sample. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of Sulfamerazine can be calculated.

Kit Components

1. 96-well microplate
2. Standards (1 ml each): 0 ng/ml, 0.5 ng/ml, 1.0 ng/ml, 2.0 ng/ml, 4.0 ng/ml, 8.0 ng/ml
3. Detection Reagent A: 5.5 ml
4. Detection Reagent B: 5.5 ml
5. Substrate Reagent A: 6 ml
6. Substrate Reagent B: 6 ml
7. Stop Solution: 6 ml
8. Wash Buffer (20X): 40 ml
9. Reconstitution Buffer (2X): 50 ml
10. Plate sealer: 3
11. Hermetic bag: 1

Materials Required But Not Provided

1. Incubator
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. 50 ml centrifuge tubes
7. Absorbent filter papers
8. 100 ml and 1 L graduated cylinders
9. Nitrogen evaporator or water bath
10. Homogenizer
11. Deionized water

Reagents Required But Not Provided

1. Hydrochloric acid (HCl; 37% w/w)
2. Acetonitrile (CH_3CN)
3. Ethyl Acetate
4. Sodium hydroxide (NaOH)
5. N-hexane (C_6H_{14})
6. Hydrated Disodium phosphate (Na_2HPO_4-$12H_2O$)
7. Hydrated Monosodium phosphate (NaH_2PO_4-$2H_2O$)

Protocol

Bring all reagents and samples to room temperature before use.

A. Preparation of Samples and Reagents

1. Samples

- **0.2 M NaOH Solution** (for **Honey** samples)

Dissolve 0.8 g NaOH in 100 ml Deionized water. Mix thoroughly.

- **0.02 M PB Solution** (for **Milk** and **Muscle** samples)

Dissolve 2.58 g Na_2HPO_4-$12H_2O$ with 0.44 g NaH_2PO_4-$2H_2O$ in 500 ml Deionized water. Mix thoroughly.

- **0.5 M HCl Solution** (for **Honey** samples)

Dilute 4.3 ml Concentrated HCl with 95.7 ml Deionized water.

- **1X Reconstitution Buffer** (for **Honey, Egg,** and **Muscle** samples)

Dilute the provided 2X Reconstitution Buffer 2-fold with Deionized Water (i.e. in a 1:1 ratio of 2X Reconstitution Buffer to Deionized water).

- **Wash Buffer (1X)**

Dilute the provided 20X Wash Buffer 20-fold with Deionized Water (i.e. in a 1:19 ratio of 20X Wash Buffer to Deionized water).

2. Samples

- **Milk:** Add 20 µl of Milk sample into 380 µl 0.02 M PB Solution and mix fully. Take 50 µl of the resulting solution for analysis.
  Note: Sample dilution factor: 20; Detection limit: 10 ng/ml.

- **Honey:** Weigh 1 g of Honey homogenate into a centrifuge tube and add 1 ml 0.5 M HCl Solution. Vortex for 1 minute, then incubate at 37°C for 30 minutes. Add 2.5 ml of 0.2 M NaOH Solution, adjust pH to 5. Add 4 ml of Ethyl Acetate, vortex for 5 minutes, then centrifuge at 4000 rpm for 10 minutes. Take 2 ml of the resulting supernatant into a fresh centrifuge tube, and dry at 50 – 60°C in a nitrogen evaporator or water bath. Dissolve the dry residue with 0.5 ml of 1X Reconstitution Buffer and vortex for 1 minute, then centrifuge at 4000 rpm for 5 minutes. Aliquot 50 µl of the liquid for analysis. Analyze immediately.
  Note: Sample dilution factor: 1; Detection limit: 0.5 ng/ml.

- **Eggs:** Homogenize the Egg sample. Weigh 2 g of homogenate into 4 ml of Ethyl Acetate, and vortex fully for 2 minutes. Centrifuge at 4000 rpm for 10 minutes. Take 2 ml of the supernatant and dry at 50 - 60°C in a nitrogen evaporator or a water bath. Dissolve the dry residue with 2 ml of N-Hexane and 0.5 ml of 1X Reconstitution Buffer and vortex for 4 minutes, then centrifuge at 4000 rpm for 5 minutes. Aliquot 50 µl of the liquid for analysis. Analyze immediately.
  Note: Sample dilution factor: 0.5; Detection limit: 0.25 ng/ml.

- **Muscle:** Remove as much fat as possible from the sample, then homogenize and mix fully. Weigh 3 g of homogenate into 3 ml of 0.02 M PB Solution in a centrifuge tube, and vortex strongly until the sample is fully dissolved. Add 4 ml Ethyl Acetate and 2 ml Acetonitrile, then vortex for 10 minutes. Centrifuge at 4000 rpm for 10 minutes. Take 2 ml of the uppermost liquid layer and dry at 50 - 60°C in a nitrogen evaporator or a water bath. Add 1 ml N-hexane and 1 ml of 1X Reconstitution Buffer (Solu to dissolve the dry material. Vortex for 1 minute, then centrifuge at 4000 rpm for 5 minutes. Aliquot 50 µl of the lower liquid layer for analysis. Analyze immediately.
  Note: Sample dilution factor: 1; Detection limit: 0.5 ng/ml.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Number the sample and standard wells, and record their positions. *All samples and standards should be tested in duplicate.*
2. Add 50 µl of standard or sample into the respective standard and sample wells.
3. Add 50 µl Detection Reagent B and then add 50 µl Detection Reagent A to all wells.
4. Cover the plate with a plate sealer, shake gently for 5 seconds to mix, and incubate at 25°C for 45 minutes in the dark.
5. Remove the cover, and wash the plate 5 times. *Discard the solution without touching the side walls. Blot the plate on an absorbent material. Add 300 µl of wash buffer to each well and soak for at least 30 sec. Discard the contents and clap the plate on absorbent filter papers or other absorbent material.*
6. Add 50 µl of Substrate Reagent A to each well.
7. Add 50 µl of Substrate Reagent B to each well. Shake gently for 5 seconds to ensure thorough mixing, then incubate at 25°C for 10-20 minutes in the dark.
8. Add 50 µl of Stop Solution to each well and shake gently to ensure thorough mixing.
9. Read and record the absorbance of each well at 450 nm with a microplate reader. The absorbance must be measured within 10 minutes of introducing the Stop Solution.

C. Analysis of Results

This assay is competitive, therefore there is an inverse correlation between Sulfamerazine concentration in the sample and the absorbance measured. The absorbance (%) should be calculated as follows:

$$\%\ \text{Absorbance} = \frac{A}{A_0} \times 100$$

where:

A	Average absorbance of the sample well
A_0	Average absorbance of the blank (0 ng/ml) well

Plot the Absorbance (%) on the y-axis, and the log concentration (ng/ml) on the x axis. Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

D. Precautions

Bring all reagents to room temperature prior to use.

1. Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.
2. Do not allow the wells to dry fully during the washing procedure. Ensure the Wash Buffer is thoroughly mixed, and the wells are washed completely.
3. Do not use the Substrate Reagent if it has begun to turn blue without being added to the wells.
4. Do not use any reagents that are expired, or use reagents from other kits with this assay.
5. The Stop Solution is caustic. Avoid contact with the skin or eyes, and thoroughly wash any spilled solution with water.
6. It is recommended that the same operator perform the experiment throughout, to avoid inconsistencies between operator procedures.