Sulfonamides (Sas) Assay Kit

Catalog No.: abx364900

Size: 96 tests

Storage: Store all components at 4°C.

Application: For detection and quantification of Sulfonamides in samples such as serum, honey, muscle tissue, milk, and feed.

Introduction

Sulfonamides are a collection of synthetic medication primarily used as antibiotics. They all contain the Sulfonamide functional group and work by mimicking the action of PABA and inhibiting the production of bacterial folic acid, limiting their growth and reproduction. They are commonly used to treat urinary tract infections, bronchitis and other inefections.

This kit is based on competitive enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. The standards, samples, HRP-conjugated reagent, and a biotin-conjugated antibody specific to Sulfonamides are added to the wells and incubated. After washing away the unbound conjugates, TMB substrate is added. Only wells that contain Sulfonamides 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 Sulfonamides amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of Sulfonamides can be calculated.

Kit components

1. 96-well microplate
2. Standards (0 ng/ml, 2 ng/ml, 6 ng/ml, 18 ng/ml, 54 ng/ml, 162 ng/ml): 1 ml each
3. Detection Reagent A: 10 ml
4. Detection Reagent B: 7 ml
5. Substrate A: 6 ml
6. Substrate B: 6 ml
7. Stop Solution: 6 ml
8. Wash Buffer (20X): 25 ml
9. Sample Diluent (20X): 50 ml
10. Plate sealer: 3
11. Hermetic bag: 1

Materials required but not provided

- Microplate reader (450 nm)
- Deionized water
- Pipette and pipette tips
- Microcentrifuge tubes
- Centrifuge
- Nitrogen evaporator or water bath
- Homogenizer
- Vortex mixer
- Incubator
- Balance

Reagents required but not provided

1. Potassium Ferrocyanide ($K_4Fe(CN)_6 \cdot 3H_2O$)
2. Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$)
3. di-Sodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)
4. Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$)
5. Trichloroacetic Acid ($C_2HCl_3O_2$)
6. Sodium Hydroxide (NaOH)
7. Concentrated Phosphoric Acid (H_3PO_4)
8. Acetonitrile
9. N-hexane
10. Methanol

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Potassium Ferrocyanide Solution**

Dissolve 1.52 g of potassium ferrocyanide ($K_4Fe(CN)_6 \cdot 3H_2O$) in 10 ml of deionized water an mix thoroughly.

- **Zinc Sulfate Solution**

Dissolve 2.88 g of zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) in 8.64 ml of deionized water and mix thoroughly.

- **PB Solution**

Dissolve 6 g of di-sodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) and 0.5 g of Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$) in 300 ml of deiomized water and mix thoroughly.

- **Extraction Solution**

Dissolve 1 g of trichloroacetic acid (C_2HCl_3O2) in 100 ml of deionized water and mix thoroughly.

- **1 M Sodium Hydroxide Solution**

Dissolve 4 g of sodium hydroxide (NaOH) in 100 ml of water and mix thoroughly.

- **Phosphoric Acid Solution**

Add 2 ml of concentrated phosphoric acid (H_3PO_4) into 98 ml of deionized water and mix thoroughly.

- **Sample Diluent**

Dilute the Sample Diluent (20X) 20 fold using deionized water to a ratio of 1:3. For example add 1 ml of Sample Diluent (20X) to 3 ml of deionized water to produce 4 ml of Sample Diluent.

- **1X Wash Buffer**

Dilute the Wash Buffer (20X) using deionized water to a ratio of 1:19. For example add 1 ml of Wash Buffer (20X) to 19 ml of deionized water to produce 20 ml of 1X Wash Buffer.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- Prepare reagents in accordfance to the number of samples used. Reagents do not all need to be used at once.

2. Sample Pretreatment

- **Urine (Pig):** If sample is cloudy, centrifuge at 4000 rpm for 5 minutes or longer if required to clarify sample. Carefuly collect 20 µl of supernatant and assay immediately.

  Note: Sample dilution factor: 1, Detrection Limit: 40 ng/ml.

- **Milk (raw and pasteurized):** Add 1 ml of sample to 100 µl of Zinc Sulfate Solution and 100 µl of Potassium Ferrocyanide Solution. Vortex sample for 30 seconds to mix thoroughly. Add 1.8 ml of PB Solution and centrifuge at 4000 rpm for 5 minutes at room temperature. Carefully collect 200 µl of supernatant and add to 200 µl of deionized water. Mix thoroughly and collect 20 µl for immediate analysis.

  Note: Sample Dilution Factor: 6, Detection Limit: 20 ng/ml.

- **Milk Powder:** Weigh 1 ± 0.05 g of sample and add to 8 ml of deionized water. Vortex for 30 seconds to mix thoroughly. Add 1 ml to 100 µl of Zinc Sulfate Solution and 100 µl of Potassium Ferrocyanide Solution. Vortex sample for 30 seconds to mix thoroughly. Add 1.8 ml of PB Solution and centrifuge at 4000 rpm for 5 minutes at room temperature. Carefully collect 200 µl of supernatant and add to 200 µl of deionized water. Mix thoroughly and collect 20 µl for immediate analysis.

  Note: Sample Dilution Factor: 6, Detection Limit: 20 ng/ml.

- **Yogurt:** Weigh 1 ± 0.05 g and adjust pH to 7 using 1 M Sodium Hydroxide Solution. Add 1 ml of sample to 100 µl of Zinc Sulfate Solution and 100 µl of Potassium Ferrocyanide Solution. Vortex sample for 30 seconds to mix thoroughly. Add 1.8 ml of PB Solution and centrifuge at 4000 rpm for 5 minutes at room temperature. Carefully collect 200 µl of supernatant and add to 200 µl of deionized water. Mix thoroughly and collect 20 µl for immediate analysis.

  Note: Sample Dilution Factor: 6, Detection Limit: 20 ng/ml

- **Serum:** Add 1 ml of sample to 1 ml of methanol and 1 ml of 1X Wash buffer. Vortex for 1 minute to mix thoroughly. Centrifuge at 4000 rpm for 5 minutes at room temperature. Carefully collect 20 µl of supernatant for immediate analysis.

  Note: Sample dilution factor: 1, Detrection Limit: 40 ng/ml.

- **Muscle (Pork, Fish, Shrimp, Chicken, Beef, Mutton and Duck):** Remove the fat from the sample and homogenize. Weigh 1 ± 0.05 g of homogenate and add to 9.5 ml of deionized water and 500 µl of Sample Diluent, then vortex for 1 minute. Centrifuge at 4000 rpm for 10 minutes at room temperature. Collect 20 µl of the supernatant for analysis.

  Note: Sample Dilution Factor: 10, Detection Limit: Pork - 40 ng/ml, Fish, chicken and shrimp - 20 ng/ml, Beef, mutton and duck - 50 ng/ml.

- **Egg:** Homogenize sample and weigh 2 ± 0.05 g. Add homogenate to 0.1 ml of Phosphoric Acid Solution and 6 ml of acetonitrile. Vortex for 2 minutes to mix thoroughly. Centrifuge at 4000 rpm for 5 minutes at room temperature. Carefully collect 2 ml of supernatant and dry at at 60 – 70 °C using a nitrogen evaporator or water bath. Dissolve the residue in 1 ml of n-hexane and vortex for 30 seconds to mix thoroughly. Add 500 µl of Sample Diluent and vortex for 30 seconds. Centrifuge at 4000 rpm for 5 minutes at room temperature. Remove the upper and intermediate layers and collect 20 µl of the lower layer for analysis.

  Note: Sample Dilution Factor: 1, Detection Limit: 2 ng/ml.

- **Liver (Chicken and Pig):** Remove fat from the sample and homogenize. Weigh 2 ± 0.05 g of homogenate and add to 3 ml of 1X Wash Buffer and 3 ml of Extraction Solution. Vortex for 1 minute to mix thoroughly. Centrifuge at 4000 rpm for 5 minutes at room temperature. Carefully collect 1 ml of the intermediate layer and add to 20 µl of 1 M Sodium Hydroxide and vortex for 10 seconds to mix thoroughly. Centrifuge at 4000 rpm for 5 minutes at room temperature. Carefully collect 20 µl of supernatant for immediate analysis.

  Note: Sample Dilution Factor: 4, Detection Limit: 10 ng/ml.

- **Feed:** Homogenize sample and weigh 1 ± 0.05 g of homogenate. Add to 10 ml of deionized water and vortex for 1 minute to mix thoroughly. Centrifuge at 4000 rpm for 10 minutes at room temperature. Carefully collect 20 µl of supernatant for immediate analysis.

  Note: Sample Dilution Factor: 10, Detection Limit: 50 ng/ml.

- **Honey:** Weigh 2 ± 0.05 g and dissolve in 1 ml of deionized water. Vortex for 5 minutes to mix thoroughly. Add 100 µl of Phosphoric Acid Solution and 5 ml of acetonitrile. Vortex for 2 minutes to mix thoroughly. Centrifuge at 4000 rpm for 5 minutes at room temperature. Carefully collect 2 ml of supernatant and dry at at 60 – 70 °C using a nitrogen evaporator or water bath. Dissolve the residue in 1 ml of n-hexane and vortex for 30 seconds to mix thoroughly. Add 500 µl of Sample Diluent and vortex for 30 seconds. Centrifuge at 4000 rpm for 5 minutes at room temperature. Remove the upper and intermediate layers and collect 20 µl of the lower layer for analysis.

  Note: Sample Dilution Factor: 1, Detection Limit: 3 ng/ml.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Samples must be diluted so that the expected concentration falls within the kit's range. • Please bring sample slowly to room temperature.
- Sample hemolysis will influence the result. Hemolyzed specimen should not be used. • Samples that contain NaN3 cannot be detected as it interferes with HRP.
- Always use non-pyrogenic, endotoxin-free tubes for blood collection.

B. Assay Procedure

Equilibrate all reagents to room temperature prior ot use.

1. Assign and record well locations for each standard and sample. *It is strongly recommended to prepare all the wells in duplicate.*
2. Add 20 µl of sample to each sample well.
3. Add 20 µl of each standard to the corresponding standard wells.
4. Immediately add 50 µl of Detection Reagent B followed by 80 µl of Detection Reagent A to each well.
5. Cover the plate with a plate sealer and tap gently for 10 seconds to ensure thorough mixing.
6. Incubate the plate at 25°C for 30 minutes in the dark.
7. Remove and discard the liquid. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with 1XWash Buffer (260 µl) using a multi-channel pipette or automated washer (30 second soaking period is recommended).Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
8. To each well, add 50 µl of Substrate A followed by 50 µl of Substrate B.
9. Cover the plate with a plate sealer and tap gently for 10 seconds to ensure thorough mixing.
10. Incubate the plate at 25°C for 15 minutes in the dark.
11. Add 50 µl of Stop Solution to each well and. It is important the Stop Solution is mixed quickly an uniformly throughout the microplate to inactivate the enzyme completely.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measurethe absorbance at 450 nm immediately using a microplate reader.

C. Calculation of Results

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

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

where:

$\boldsymbol{A}$	Average absorbance of standard/sample
A_0	Average absorbance of 0 ng/ml standard

Plot the absorbance (%) of the standard dilutions on the y-axis, and their corresponding concentrations (ng/ml) on the x axis. Apply a best fit trendline through the standard points. Use this graph calculate sample Sulfonamide concentrations based on their absorbance (%) 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. Cross Reactivity

	Observed Cross Reactivity
Sulfamethoxazole(SMZ)	100 %
Sulfadiazine (SD)	22%
Sulfamethazine (SM2)	40%
Sulfamerazine(SM1)	49%
Sulfaquinoxaline(SQX)	63%
Sulfamonomethoxine (SMM)	> 100%
Sulfadimethoxine (SDM)	> 100%
Sulfathiazole(ST)	51%
Sulfamethoxypyridazine(SMP)	> 100%
Sulfapyridine(SPD)	> 100%
Sulfametoxydiazine (SMD)	> 100%
Sulfachloropyridazine(SCP)	38%
Sulfanitran (SNT)	> 100%
Sulfisomidine(SIM)	90%
Sulfamethythiadiazole (SMT)	40%

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.