Sulfonamides (SAs) ELISA Kit

Catalog Number. CSB-E12094f

This immunoassay kit allows for the in vitro quantitative determination of total sulfonamides concentrations in milk, tissue (chicken, pork, liver), swine urine.

This package insert must be read in its entirety before using this product.

If You Have Problems

Technical Service Contact information

Phone: 86-27-87582341
Fax: 86-27-87196150
Email: tech@cusabio.com
Web: www.cusabio.com

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

INTRODUCTION

Sulfonamides are widely used in animal industry and play an important role in controlling and treatment livestock and poultry diseases. Sulfonamides have become a threat for human health and affected the export of animal derived food due to Sulfonamides residues for abuse、not abiding by withdrawal time. Sulfonamides residues limit is 100 mg/kg according to No.235 document of the Ministry of Agriculture of the People's Republic of China. The method of instrumental analytical is major to detect Sulfonamides residues, but it needs expensive instruments, professional operators and complex pre-treatment; While the method of enzyme linked immunoassay has advantages with simple, rapid, high sensitivity, good specificity and low cost.

This kit is a detection product developed based on ELISA technology, with operation time as short as 50 min and a sensitivity of 2 ppb, and linear range from 2 ppb to 162 ppb.

PRINCIPLE OF THE ASSAY

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with Sulfonamides antigen. Standards or samples are added to the appropriate microtiter plate wells with Sulfonamides specific antibody and Horseradish Peroxidase (HRP) conjugated anti-antibody. The competitive inhibition reaction is launched between pre-coated Sulfonamides and Sulfonamides in standards or samples with the Sulfonamides special antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of Sulfonamides in the standards or samples. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

2 ppb-162 ppb

SENSITIVITY

The minimum detectable dose of the kit is typically less than 2 ppb.

The sensitivity of this assay, or Lower Limit of Detection (LOD) was defined as the lowest concentration that could be differentiated from zero. It was determined the mean OD value of 20 replicates of the zero standard added by their three standard deviations.

CROSS-REACTION RATE

Sulfadimethoxine (SDM)	100%
Sulfamonomethoxine (SMN)	134%
Sulfamerazine (SM1).	148%
Sulfadimidine	343%
Sulfametoxydiazine (SMD)	328%
Sulfaquinoxaline (SQX)	57%
Sulfadiazine(SD)	45%

RECOVERY RATE

Swine urine	70%-100%
Milk	70%-100%
Tissue	70%-100%

LIMIT OF DETECTION

Swine urine	2 ppb
Milk	20 ppb
Tissue	20 ppb

PRECISION

Intra-assay Precision (Precision within an assay): CV%<10%
Three samples of known concentration were tested twenty times on one plate to assess.
Inter-assay Precision (Precision between assays): CV%<10%
Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Reagent	Quantity(96T)	Quantity(48T)
Assay plate	96 wells	48 wells
Standard	6 x 1 mL	6 x 0.5 mL
HRP-conjugate	1 x 7 mL	1 x 3.5 mL
Antibody	1 x 7 mL	1 x 3.5 mL
TMB Substrate	1 x 12 mL	1 x 6 mL
Stop Solution	1 x 10 mL	1 x 5 mL
Sample Diluent(5×)	1 x 20 mL	1 x10 mL
Wash Buffer(10×)	1 x 50 mL	1 x 25 mL
Adhesive Strip	4	4
Instruction Manual	1	1

STANDARD CONCENTRATION

Standard	S0	S1	S2	S3	S4	S5
Concentration (ppb)	0	2	6	18	54	162

STORAGE

Unopened kit	Store at 2 - 8°C. Do not use the kit beyond the expiration date
Opened kit	May be stored for up to one month at 2 - 8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- An incubator which can provide stable incubation conditions up to 25°C
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Centrifuge, Vortex mixer
- Analytical balance, 2 decimal place
- Absorbent paper for blotting the microtiter plate.
- Single-channel micropipette(20 μL-200 μL、100 μL-1000 μL)
- 300 μL multichannel micropipette
- 100 mL and 500 mL graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.
- $Na_2HPO_4.12H_2O$
- $NaH_2PO_4.2H_2O$

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Note:

- Kindly use graduated containers to prepare the reagent.
- Bring all reagents to room temperature (20-25°C) before use for 30 min.
- Only the disposable tips can be used for the experiments and the tips must be changed when used for different reagents.
- Distilled water is recommended to be used to make the preparation for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature (20~25℃) before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 2-8°C.
3. Add 50 µL of **Standard** or **Sample** per well. Then add 50 µL of **HRP-conjugate** to each well and 50 µL **Antibody** to each well. Cover the microtiter plate with a new adhesive strip and mix well, then incubate for 30 min at 25°C.
4. Aspirate each well and wash, repeating the process four times. Wash by filling each well with 250 µL of **Wash buffer (1x)** using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 30 seconds, complete removal of liquid at each step is essential to good performance.
5. Add 100 µL of **TMB Substrate** to each well, mix well. Incubate for 15 minutes at 25°C. Protect from light.
6. Add 50 µL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well within 5 min, using a microplate

reader set to 450 nm (Recommend to read the OD value at the dual-wavelength: 450/630 nm within 5 min).

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 min. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding Substrates (e.g. observation once every 10 min). Substrates should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. Substrates are easily contaminated. Substrates should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the

Substrates. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrates.

CALCULATION OF RESULTS

There are two methods to judge the results: the first one (A) is the rough judgment, while the second (B) is the quantitative determination. Note that the OD value of the sample has a negative correlation with Sulfonamides in the sample.

A:

Compare the sample average absorbance values with standards values, the Sulfonamides concentration in the samples can be concluded. For example, the absorbance value of sample 1 is 0.353, the absorbance value of sample 2 is 1.429; absorbance values of standard are:1.926, 1.579, 1.448, 0.711, 0.159, 0.094 and the corresponding concentrations are:0 ppb, 2 ppb, 6 ppb, 18 ppb, 54 ppb, 162 ppb; then the Sulfonamides in sample 1 and sample 2 are 18 ppb-54 ppb and 6 ppb-18 ppb; Lastly the reader is multiplied by the corresponding dilution factor of each sample and the actual concentration of sample is obtained

B:

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbency value}(\%) = \frac{B}{B_0} \times 100\%$$

B ——the average absorbance value of the sample or standard

B_0 ——the average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Sulfonamides standards solution (ppb) as x-axis.

The Sulfonamides concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained. (The software offered together will facilitate the calculation process, it's suitable for accurate and fast analysis of large scale samples, please contact us)

Note:

- Discard the substrate with any color that indicates the degeneration of this solution; when the absorbance value of standard solution 0 of less than 0.5 indicates its degeneration.
- The optimum reaction temperature is 25℃, and too high or too low will result in the changes in the absorbance value and detecting sensitivity.