Sulfonamides ELISA Kit

Product #: E6203, 96T

For research use, for professional use. Not for IVD or therapeutic purpose.

Manufacturer Information

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1. Description

Sulfonamides represent a large class of antibiotics that have multiple clinical uses. They are considered bacteriostatic and appear to act by inhibition of bacterial biosynthesis of folic acid, which is needed for cell growth, at least in those bacteria that are sensitive to Sulfonamides. Now in all major countries, strict maximum residue limits have been set on Sulfonamides. In EU, the total residue of Sulfonamides is set at 100ug/kg for milk, meat, etc.

This current Sulfonamides ELISA Kit is based on indirect competitive ELISA to detect Sulfonamides residue in milk and honey. In the kit, sulfa antigen is coated on the microtiter well and samples contained the drug will compete for Sulfonamides specific antibody with the antigen. After washing and adding the HRP conjugate, TMB substrate is used to show the reaction color. The concentration of Sulfonamides in sample is inversely proportional to the OD value, which can be interpreted with a standard curve.

2. Application

This kit is applicable for determination of Sulfonamides residue in milk, milk powder and meat. Other applications can also be developed upon request.

3. Kit components

1) Microtiter plate, 96wells, 1 plate
2) Sulfonamides standards, 1mL/vial, 6 vials, 0, 0.2, 0.5, 1.0, 2.0, and 4.0 ng/mL
3) Spiking standard, 1 mL/vial, 1000ng/mL
4) Antibody solution, 7mL, with green cap
5) Enzyme conjugate, 12mL, with brown cap
6) TMB Substrate, 12mL, with brown cap
7) Stop solution 7 mL – **Not provided in the kit due to air cargo shipment restriction**.
8) 20x Wash buffer, 50mL, with transparent cap
9) 5x Sample buffer, 60mL, with transparent cap
10) 2x Tissue buffer, 15mL, with transparent cap
11) Cover membrane, 2 pieces

4. Instrument and material required

1) ELISA reader, with 450/630nm
2) Centrifuge
3) Balance, 0.01g
4) Centrifuge tube, 2mL, 10mL
5) Vortex mixer
6) Micropipette, 20-200, 100-1000μL
7) Multi-channel pipette, 250μL
8) graduated pipette,10mL

5. Reagent required

Deionized water

6. Buffer preparation

Buffer 2 is used for milk sample, and buffer 3 is used for milk powder sample, buffer 4 is used for meat sample.

Buffer 1: Wash buffer:

Dilute 20x wash buffer with deionized water, in the volume ratio of 1:19, for example, 10mL 20x wash buffer + 190mL deionized water, mix thoroughly.

This diluted wash buffer can be stored at 2-8 ◦C for 1 month.

Buffer 2: 1x sample buffer

Dilute 5x sample buffer with deionized water, in the volume ratio of 1:4, for example, 10mL 5x sample buffer + 40mL deionized water, mix thoroughly. Which can be used in the dilution of milk. This 1x sample buffer can be stored at 2-8 ◦C for 1 month.

Buffer 3: 0.5x sample buffer

Dilute 5x sample buffer with deionized water, in the volume ratio of 1:9, for example, 10mL 5x sample buffer + 90mL deionized water, mix thoroughly. Which can be used in the dilution of milk powder.

This 0.5x sample diluent can be stored at 4◦C for 1 month.

Buffer 4: 1x tissue buffer

Dilute 2x tissue buffer with deionized water, in the volume ratio of 1:1, for example, 10mL 2x sample buffer + 10mL deionized water, mix thoroughly. Which can be used in the dilution of meat sample.

This 1x tissue diluent can be stored at 2-8 ◦C for 1 month.

Stop solution: 0.5-2M sulfuric acid, not provided in the kit. Customer shall prepare in their own lab.

7. Sample preparation

7.1 Precautions before prepare samples:

1) Use disposable tips during the test. Change new tip for different sample / reagent.
2) Make sure all lab wares are clean and ready to use.
3) Prepared sample shall be analyzed immediately after dilution.

7.2 Milk

Take 1.0±0.05mL raw milk into 5mL centrifuge tube, dilute with 3mL of 1x sample buffer, mix thoroughly and then take 50μL use for assay.

7.3 Milk powder

Take 1.0±0.05g sample into 15mL centrifuge tube, add 8mL deionized water, vortex for 3min to dissolve, then take 1mL dissolve milk sample into 5mL centrifuge tube, dilute with 2mL of 0.5x sample buffer, vortex for 30s to mix thoroughly. Then take 50μL for assay.

7.4 Meat

Take 1.0±0.05g meat sample into 15mL centrifuge tube, add 4mL deionized water, mix thoroughly by vortex for 2min. Centrifuge for 5min at 4000rpm, take 100μL supernate into a 2mL centrifuge tube, add 300μL of 1x tissue buffer, vortex for 10s, take 50μL for assay.

8. Notice and precautions before assay

1) Make sure the ELISA kit and all reagents are returned to room temperature (20-25 ◦C). For example, keep these reagent and kits at room temperature for at least 60min.
2) Return unused kit components to 2-8 ◦C.
3) Washing step is important for the reproducibility of the kit, please follow this instruction carefully.
4) Cover the ELISA plate during all incubation. Avoid direct sunlight.

9. Assay procedures

1) Return the ELISA kit and all reagents to room temperature (20-25 ◦C). For example, keep these reagent and kits at room temperature for at least 30min.
2) The sample buffer, sample diluent, wash buffer shall also be brought to room temperature.
3) Take needed microwells and zip rest in the zip-bag and return to 2-8 ◦C.
4) Layout the plate and record sample and standard well positions. It is recommended to run all tests in duplicates.

5) **Add sample/standard/antibody solution**: add sample/standard into the wells, 50μL per each, then add antibody solution, 50μL per well, shake gently and then cover the plate and incubate at **25 °C for 30min**.
6) **Wash**: take out the plate and pour the liquid out. Use the diluted wash buffer (**buffer 1**) to wash the plate, 250μL/well. Wash for 4-5 times with interval of 10s. The pour the liquid out and tap the plate against absorbent paper. Eliminate the air bubble in the wells with micropipette tip if the bubble exists.
7) **Add Enzyme conjugate**: add enzyme conjugate, 100μL per well, shake gently and then cover the plate and incubate at **25 °C for 30min**.
8) **Wash:** wash the plate again.
9) **Coloration**: add TMB substrate, 100μL per well, and then cover the plate and incubate **25 °C for 15min**.
10) **Stop the reaction**: add stop solution, 50μL per well, shake gently and read the plate with ELISA reader at **450nm**. Read the plate within 5min after adding stop solution.

10. Result Calculation

10.1 Qualitative estimation

This kit is based on competitive ELISA, thus the OD values is inversely proportional to the Sulfonamides content contained in sample. If there is no ELISA reader, just compare the color of sample with the Sulfonamides Standard to get the estimated sample Sulfonamides content.

10.2 Quantitative calculation

Calculation of absorbance: the percentage absorbance of standard product or sample is equal to the average absorbance value of standard product or sample (double hole) divided by the average absorbance value of blank standard product, and then multiplied by 100%, i.e

$$\text{percentage absorbance } (\%) = \frac{B}{B_0} \times 100\%$$

B - the average absorbance value of standard product or sample

B_0- the average absorbance value of blank standard product

10.3 Standard curve

The standard curve was drawn by taking the percentage absorption of standard substance as the vertical coordinate and the concentration of standard substance sulfonamides as the horizontal coordinate. Put the percentage absorption rate of samples into the standard curve, read the corresponding concentration of samples from the standard curve, and multiply the corresponding dilution times to obtain the actual concentration of sulfonamides in samples.

11. Sample dilution factor:

Milk: 4

Milk powder: 3

Meat: 20

12. Specifications of the kit

1) **Sensitivity**: 0.2ng/mL
2) **Specificity**:

Sulfamethazine: 100%;

Sulfadimethoxine: 41%;

Sulfametoxydiazine: 90%;

Sulfamerazine: 83%;

Sulfadiazine: 23%;
Sulfapyridine: 4%;
Sulfadimethylisoxazole: 25%;
Sulfaquinoxaline: 52%;
Sulfabenzamide: 10%;
Sulfamethizole: 10%;
Sulfamoxole, sulfanitran, sulfisoxazole, sulfaphenazole, sulfanaidine, sulfathiazole: less than 1%;

3) **Limit of Detection**:

Milk: 0.8 ng/mL
Milk powder: 0.6ng/mL
Meat: 4 ng/mL

4) **Recovery**: 70%-120%
5) **Precision**: C.V<10%.

13. Cautions and tips for the test

1) Lower room temperature, e.g., lower than 20 ◦C may cause lower OD values. Please make sure all reagent and kit components are returned to room temperature.
2) Wash step is vital for the reproducibility of the kit. Please wash according to the kit instruction. Do not let the plate dry during wash. Continue the next operations immediately after wash step.
3) Shake each reagent gently before use.
4) Stop solution is acidic, please handle with care.
5) Do not use expired kits and reagents. Do not mix the reagent and kits from different LOT.
6) The kit is stored at 2-8◦C(36-46◦F), do not freeze.
7) TMB substrate is sensitive to sunlight. Avoid direct sunlight.
8) If Standard 1 (0ng/L) OD is lower than 0.5, please do not use. The kit may be expired or deteriorated.
9) The coloration step takes 15min. You can prolong it to 20min-25min if the color of the well is too light. On the contrary, please reduce the incubation time.
10) The incubation is 25◦C, lower or higher temperature will cause changes of OD and sensitivity of the kits, which may affect the result of the assay.

14. Storage and expiration

The kit is valid for 12months when stored at 2-8 ◦C. LOT and Expiry information are printed on the package.