Sulfonamides residue ELISA Kit

06/16

(Catalog # K4207-100, 100 assays, Store at 4°C)

I. 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 residue for abuse, not abiding by withdrawal time. The method of instrumental analytical is major to detect Sulfonamides residue, 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 competitive ELISA technology, with operation time as short as 50 min and a sensitivity of 1 ppb, and linear range from 1 ppb to 81 ppb.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Sulfonamides residue concentrations in milk, tissue (chicken, pork, beef, liver), swine urine, egg.
Detection Range: 1 - 81 ppb
Sensitivity: 1 ppb
Detection limitation: 3 ppb for swine urine, 10 ppb for egg, 20 ppb for milk and tissue.

III. Sample Type:

Milk, tissue (chicken, pork, beef, liver), swine urine, egg

IV. Kit Contents:

Components	K4207-100	Part No.
Micro ELISA Plate	8 X 12 Strips	K4207-100-1
Standard	1 ml X 6	K4207-100-2
HRP-conjugate	7 ml	K4207-100-3
Antibody	7 ml	K4207-100-4
TMB substrate	12 ml	K4207-100-5
Stop Solution	10 ml	K4207-100-6
Sample Diluent 1	40 ml	K4207-100-7
Sample Diluent 2	15 ml	K4207-100-8
Sample Extraction (50X)	15 ml	K4207-100-9
Wash Buffer (10X)	50 ml	K4207-100-10
Plate sealers	4	K4207-100-11

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 25°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes and graduated cylinders for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Extraction Solution A (for tissue use only):** According to the required amount, dilute 5 mL of Sample Extraction (50×) into 245 mL deionized or distilled water and mix well. Keep it at 4°C for one month.
2. **Extraction Solution B (for milk and egg use only):** According to the required amount, dilute 10 mL of Sample Diluent 2 into 40 ml deionized or distilled water, then add 2.5 g Extraction Reagentand mix well.
3. **Wash Buffer (1X**): If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals are completely dissolved. Dilute 10 ml of Wash Buffer (10X) into 90 ml deionized water to prepare 100 ml of Wash Buffer (1X). Can be stored at 4°C for one month.
4. **Standards Concentration:**

Standards	S0	S1	S2	S3	S4	S5
Concentration (ppb)	0	1	3	9	27	81

5. **Sample Preparation:**

Note: The prepared sample maybe stored for up to one day at 2-8°C.

- **Tissue (pork, liver, beef, chicken)**
  1. Weigh 1 g of the homogenized sample and add 5 ml of **Extraction Solution A**, vortex for 5 min.
  2. Centrifuge at 4000 rpm for 10 min. Transfer 200 µl of supernatant into a new centrifugal tube.
  3. Add 200 µl of Sample **Diluent 1**, shake well and take 50 µl of sample for further analysis. (Dilution Factor: 10)
- **Milk and fresh milk**
  1. Bring the milk sample to room temperature.
  2. Mix 100 µl of sample with 900 µl of **Extraction Solution B** in a new centrifugal tube.
  3. Take 50 µl of sample for further analysis. (Dilution Factor: 10)
- **Egg**
  1. Weigh 1 g of the homogenized sample and add 4 ml of distilled water, vortex for 5 min.
  2. Take 500 µl of sample into 500 µl of **Extraction Solution B**, shake well. Take 50 µl of sample for further analysis. (Dilution Factor: 10)
- **Swine urine**
  1. Balance to room temperature and take 50 µl of urine for test. If the urine is not clear, centrifuge at 2000 rpm for 5 min and take supernatant for future analysis. (Dilution Factor: 1)

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 50 µl of **Standard** or **Sample** per well. Then add 50 µl of **HRP-conjugate** to each well and 50 µl of Antibody to each well. Cover the microtiter plate with a new adhesive strip and mix well, then incubate for 30 min at 25°C.
3. Aspirate each well and wash, repeating the process 4 times. Wash by filling each well with 250 µl of **Wash Buffer** 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.
4. Add 100 µl of **TMB Substrate** to each well, mix well. Incubate for 15 minutes at 25°C. Protect from light.
5. Add 50 µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
6. Read result at 450 nm within 10 minutes.

IX. CALCULATION:

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.

$$\textbf{Absorbance Value (\%)} = B/B_0 \times 100\%$$

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 Fluoroquinolones standards solution (ppb) as x-axis. The Fluoroquinolones 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.

X. RELATED PRODUCTS:

- Ampicillin sodium (Cat. No. 2484-5G, 25G, 100G)
- Carbenicillin disodium (Cat. No. 2485-1G, 5G, 10G)
- Colistin Sulfate (Cat. No. 9696-1G, 5G)
- Penicillin G sodium (Cat. No. 2503-100, 500)

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