Sulfonamides residue ELISA Kit

rev.07/21

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

II. Application:

- This ELISA kit is used for the *in vitro* quantitative determination of Sulfonamides residue concentrations in milk, honey, tissue, urine.
- **Detection Range:** 1 - 81 ppb
- **Sensitivity:** < 1 ppb
- Detection limitation: 4 ppb for urine, 1 ppb for honey, 20 ppb for milk, 10-5 ppb for tissue.

III. Sample Types:

Milk, honey, tissue, urine

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
Substrate A	7 ml	K4207-100-5
Substrate B	7 ml	K4207-100-6
Stop Solution	7 ml	K4207-100-7
Sample Redissolving Buffer (20X)	50 ml	K4207-100-8
Wash Buffer (20X)	40 ml	K4207-100-9
Plate sealers	4	K4207-100-10

V. User Supplied Reagents and Equipment:

- Ethyl Acetate, N-hexane (for tissue samples)
- Acetonitrile-dichloromethane (for tissue samples)
- NaOH
- HCl
- Na_2HPO_4-citric acid solution
- 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. Reagents and Sample Preparation:

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

1. **0.2 M NaOH:** Weight 0.8 g of NaOH into 100 ml distilled water and mix well
2. **0.5 M HCl:** Mix 4.3 ml of conc HCl with 100 ml distilled water and mix well
3. **Na_2HPO_4-citric acid solution:** Take 19.85 gm $Na_2HPO_4.12H_2O$ and 9.3 gm Citric acid monohydrate into 1000 ml distilled water and mix well.
4. **Acetonitrile-dichloromethane solution:** Mix 1 volume of Acetonitrile with 4 volumes of Dichloromethane and mix well.
5. **Sample Redissolving Buffer (1X):** Dilute 1 ml of Sample Redissolving Buffer (20X) with 10 ml distilled water and mix well

6. **Wash Buffer (1X**): If crystals have formed in the concentrate, warm up to room temperature (RT) and mix gently until the crystals are completely dissolved. Dilute 20 ml of Wash Buffer (20X) into 380 ml deionized water to prepare 400 ml of Wash Buffer (1X). Keep it at 4°C for one month.
7. **Standards Concentration:**

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

8. **Sample Preparation:**

- **Tissue (high detection limit method A, 1 ppb)**
  1. Weigh 2 g of the homogenized sample and add 6 ml of **Ethyl Acetate** and vortex for 2 min.
  2. Centrifuge at 4000 rpm for 10 min. Take 3 ml of clear organic layer. The sample can be dried by blowing nitrogen gas at 50-60 °C.
  3. Dilute the sample with 1 ml of Sample Redissolving Buffer (1X) and 1 ml of N-hexane and shake for 30 sec.
  4. Centrifuge at 4000 rpm for 5 min at 15 °C. Take 50 µl of samples for further analysis. (Dilution Factor: 1)
- **Tissue (high detection limit method B, 1 ppb)**
  1. Weigh 2 g of the homogenized sample and add 6 ml of **Acetonitrile-dichloromethane solution** and vortex for 2 min.
  2. Centrifuge at 4000 rpm for 10 min at 15 °C.
  3. Take 4 ml of the organic layer. The sample can be dried by blowing nitrogen gas at 56 °C.
  3. Dilute the sample with 1 ml of Sample Redissolving Buffer (1X) and add 1 ml of N-hexane and shake for 30 sec.
  4. Centrifuge at 4000 rpm for 5 min at 15 °C. Take 50 µl of sample for further analysis. (Dilution Factor: 1)
- **Tissue (low detection limit method B, 5 ppb)**
  1. Weigh 2 g of the homogenized sample and add 8 ml of Sample Redissolving Buffer (1X) and vortex for 2 min.
  2. Centrifuge at 4000 rpm for 10 min at 15 °C.
  3. Take 50 µl of sample for further analysis. (Dilution Factor: 5)
- **Milk**
  1. Take 20 µl of milk sample and add 380 µl of Sample Redissolving Buffer (1X). Shake for 30 sec.
  2. Take 50 µl of sample for further analysis. (Dilution Factor: 20)
- **Urine**
  1. Take 3 ml Weigh of Sample Redissolving Buffer (1X) and add 1 ml clear urine. Shake for 30 sec.
  2. Take 50 µl of sample for further analysis. (Dilution Factor: 4)
- **Honey**
  1. Weigh 1 g of the homogenized sample and add 1 ml of **0.5 M HCl** and put it at 15 °C for 30 min.
  2. Add 2.5 ml of **0.2 M NaOH** and 3 ml of **Na_2HPO_4-citric acid solution**. Then add 4 ml of **Ethyl Acetate** and vortex for 2 min.
  3. Centrifuge at 4000 rpm for 10 min at RT. Take 2 ml of organic layer. The sample can be dried by blowing nitrogen gas at 50-60 °C.
  4. Add 0.5 ml of Sample Redissolving Buffer (1X) and vortex for 30 sec.
  5. Take 50 µl of sample for further analysis. (Dilution Factor: 1)

VIII. Assay Protocol:

Note: Bring all reagents and samples to RT 30 min prior to the assay. It is recommended that all standards and samples be run at least in duplicates. 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 and 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 sec, complete removal of liquid at each step is essential to good performance.
4. Add 50 µl of **Substrate A** and 50 µl of **Substrate B t**o each well, mix well. Incubate for 15 min 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 5 min, using a microplate reader set to 450 nm. We recommend to read the OD value at the dual wavelength: 450/630 nm within 5 min).

IX. Calculation:

Note: The OD value of the sample has a negative correlation with Sulfonamides residue in the sample.
Generate the Standard Curve by plotting the average absorbance obtained for each Standard concentration on the vertical (Y) axis vs the corresponding Standard concentration (ppb) on the horizontal (X) axis. Calculate the Sulfonamides residue concentration in the samples from the Standard Curve. If the test samples were diluted, multiply the interpolated values by the dilution factor to calculate the concentration of Sulfonamides residue in the samples.

IX. Related Products:

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

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