



Data Sheet

3 Sulfonamides (SAs3) ELISA Kit

Cat. #SG-4018

Size: 96 Wells

Principle and Application

This kit adopts the method of indirect competitive enzyme-linked immunoassay (ELISA) to detect Sulfonamides (SAs) in the sample such as tissue, serum, honey, milk, egg and urine. The kit is composed of Microtiter Plate coated with coupled antigens, HRP enzyme conjugates, antibodies, standards and other supporting reagents. During the detection, with adding standards or samples, the SAs in the samples will compete with the coupled antigens to combine with anti-SAs antibodies. After adding enzyme conjugates, take coloration with TMB substrates. Absorbance value of the samples is a negative correlation with SAs content. Lastly, by comparing the obtained absorbance values with the standard curve, we can calculate the SAs content in the sample.

Storage conditions

- The kit shall be stored at 2-8 °C. Avoid freezing.
- Shelf Life: 12 months. The date of manufacture is presented in the label of the box.

Technique Data

- Kit Sensitivity: 0.1ppb (ng/mL)
- Reactive Mode: 25°C, 45min~15min
- Detection Limits:

Sample	Detection Limits
Tissue (higher detection limit)	0.1ppb
Tissue (lower detection limit)	1ppb
Serum, urine	0.4ppb
Honey	0.1ppb
Milk	2ppb
Feed	4ppb
Eggs	0.2ppb

- Cross-reaction Rate:

Drug name	Cross-reaction Rate	Sensitivity /ppb
Sulfamethoxazole(SMZ)	100%	0.5
Sulfamonomethoxine(SMM)	67%	0.15
Sulfadiazine(SD/SDZ)	33%	0.3

- Sample Recovery Rate:

Sample	Recovery Rate
Tissue, honey, egg	85±25%
Urine, milk, serum, feed	80±25%
Eggs	90±25%

Composition of the Kit

Reagent	Specification
Microtiter Plate	8wells× 12strips
Standard: 0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb	1.0mL each
High Standard: 1ppm(red cap)	1×1.0mL
Antibody solution (blue cap)	1×5.5mL
HRP conjugate (red cap)	1×5.5mL
Substrate Reagent A (white cap)	1×6mL
Substrate Reagent B (black cap)	1×6mL
Stop Solution (yellow cap)	1×6mL
Concentrated Wash Buffer (20×)(white cap)	1×40mL
Concentrated Reconstitution Buffer (2×) (yellow cap)	1×50mL
Instruction	1
Adhesive Membrane	1

Sealed bag	1
------------	---

Materials Required but Not Supplied

- **Equipment:** microplate reader, printer, grinder (for homogenizing solid samples), nitrogen evaporator, vortex mixer (**for shake and mix**), centrifuge, graduated transfer pipette, and balance with a division value of 0.01 g, constant temperature device);
- **Micropipette:** single-channel (20-200 μ L and 100-1000 μ L), and multi-channel 300 μ L;
- **Reagents:** Ethyl acetate, n-hexane, acetonitrile, $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, NaOH, concentrated hydrochloric acid (36% by mass), $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$.

Experimental preparation

Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use. This is a crucial step to ensure there is no precipitation in the reagents.

Please note that the labware must be clean. Use disposable pipette tips to avoid contamination of interference results.

◆ Solution preparation:

Solution 1: 0.1M Phosphate Buffer

Weigh 25.8g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ and 4.4 of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$,

And dissolve in deionized water and mix thoroughly. Bring the volume up to 1000mL.

Solution 2: Acetonitrile-ethyl acetate solution

Take 50 mL of acetonitrile and 50 mL of ethyl acetate and add them to a 100 mL glass bottle, then mix well.

Solution 3: 0.5 M hydrochloric acid solution

Dilute 4.3 mL concentrated hydrochloric acid to 100 mL with deionized water, mix thoroughly.

Solution 4: 0.2M NaOH Solution

Weigh 0.8g of NaOH, dissolve in deionized water, mix thoroughly, and bring the volume up to 100mL.

Solution 5: Reconstitution Buffer

Dilute the Concentrated Reconstitution Buffer (2×) 2 times (Concentrated Reconstitution Buffer (2×) /Deionized water= 1:1) .The Reconstitution Buffer can be stored for one month at 4°C.

Solution 6: Working Wash Buffer

Dilute the concentrated wash buffer (20×) by a factor of 20,
(Concentrated wash buffer/Deionized water= 1: 19)

◆ **Sample pretreatment steps:**

1. Tissue (higher detection limit) treatment.

- 1) Weigh 2 g ± 0.05 g of homogenized tissue sample and place it in a centrifuge tube. Add 1 mL of **0.1 M Phosphate Buffer (Solution 1)**, shake the sample to form a paste. Then add 7 mL of **acetonitrile-ethyl acetate solution (Solution 2)**, shake for 2 minutes. Centrifuge at 4000 r/min for 5 minutes at room temperature.
- 2) Transfer 4mL of the upper layer liquid and evaporate it to dryness under nitrogen or air at 50°C-60°C.
- 3) Add 1 mL of **n-hexane**, then add 1 mL of **Reconstitution Buffer (Solution 5)**, shake for 1 minute, centrifuge at 4000 rpm for 5 minutes at room temperature.
- 4) Remove the upper layer. Take 50µL of the lower layer for analysis.

Dilution times of the sample:1 Detection limits: 0.1ppb

2. Tissue (lower detection limit) treatment.

1) Weigh $1\text{g} \pm 0.05\text{g}$ of homogeneous tissue into a centrifuge tube. Add 10mL of **0.1M Phosphate Buffer (Solution 1)** and shake for 5 minutes. Centrifuge at 4000rpm for 5 minutes at room temperature.

2) Take 50 μL of the upper layer for analysis.

Dilution times of the sample:10 Detection limits: 1ppb

3. Egg treatment.

1) Mix egg whites and egg yolks thoroughly.

2) Weigh $2\text{g} \pm 0.05\text{g}$ of homogenized sample into a 50 mL centrifuge tube. Add 8 mL of **acetonitrile**, immediately shake for 10 minutes. Centrifuge at 4000 rpm for 5 minutes at room temperature.

3) Transfer 2mL of the supernatant to a 10mL clean and dry glass test tube, and evaporate it to dryness under nitrogen or air at 50°C - 60°C .

4) Add 1 mL of n-hexane to dissolve the dried residue, shake for 30 seconds. Then add 1 mL of **Reconstitution Buffer (Solution 5)**, shake for 1 minute. Centrifuge at 4000 r/min at room temperature for 5 minutes.

4) Remove the upper layer and take 50 μL of the lower liquid for analysis.

Dilution times of the sample:2 Detection limits: 0.2ppb

4. Serum treatment.

1) Allow the blood sample to stand at room temperature for 30 minutes, then centrifuge at 4000rpm for 10 minutes to separate the serum.

2) Take 1 mL of serum, add 3 mL of **0.1M Phosphate Buffer (Solution 1)**, and shake for 30 seconds.

3) Take 50 μL for analysis.

Dilution times of the sample:4 Detection limits: 0.4ppb

5. Honey treatment.

- 1) Weigh $1\text{g} \pm 0.05\text{g}$ of honey sample into a 50mL centrifuge tube. Add 1mL of **0.5M hydrochloric acid solution (Solution 3)** and incubate at 37°C for 30 minutes.
- 2) Add 2.5mL of **0.2M NaOH Solution (Solution 4)**, then add 4mL of **ethyl acetate**, shake for 5 minutes, and centrifuge at 4000rpm for 10 minutes at room temperature.
- 3) Take 2mL of the upper layer liquid and evaporate under nitrogen or air at 50°C . Add 0.5mL of **Reconstitution Buffer (Solution 5)**, and shake for 30 seconds.
- 4) Take 50 μL for analysis.

Dilution times of the sample:1 Detection limits: 0.1ppb

6. Urine treatment

- 1) Mix 1 mL of the clarified urine sample with 3 mL of **0.1M Phosphate Buffer (Solution 1)** and shake for 30 seconds.
- 2) Take 50 μL for analysis.

Dilution times of the sample:4 Detection limits: 0.4ppb

7. Milk treatment.

- 1) Dilute the milk sample 20 times with **0.1M Phosphate Buffer (Solution 1)** by adding 100 μL of milk to 1.9 mL of **0.1M Phosphate Buffer (Solution 1)**. Shake the mixture for 30 seconds.
- 2) Take 50 μL for analysis.

Dilution times of the sample:20 Detection limits: 2ppb

8. Feed treatment.

- 1) Weigh $2\text{g} \pm 0.05\text{g}$ of homogenized feed sample and place it in a 50 mL centrifuge tube. Add 8 mL of acetonitrile, immediately shake for 5 minutes. Centrifuge at 4000 rpm for 5 minutes at room temperature.
- 2) Transfer 1mL of the upper layer liquid to a clean, dry 10 mL container and evaporate it to dryness under nitrogen or air at 50°C - 60°C .

- 3) Add 1 mL of **n-hexane**, shake for 30 seconds. Then add 1 mL of **0.1M Phosphate Buffer (Solution 1)**, shake for another 30 seconds, then transfer into a 2mL polystyrene centrifuge tube and centrifuge at 4000 rpm for 5 minutes at room temperature.
- 4) Remove the upper layer. Transfer 100 µL of lower layer into a 2 mL centrifuge tube. Add 900 µL of **0.1M Phosphate Buffer (Solution 1)** and shake 1 minute to mix thoroughly.
- 5) Take 50µL for analysis.

Dilution times of the sample:40 Detection limits: 4ppb

ELISA procedure

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

Step 1: Number: Number the wells in sequence corresponding to the samples and standard, make 2-well parallel trials for each sample and standard, and record their locations.

Step 2: Incubation: Add 50µL of **standard or sample** into each numbered well, then add 50µL of **HRP conjugate** per well. Next, add 50µL of **antibody solution** into each well. Finally, cover the Microtiter Plate with the adhesive membrane, shake gently by hand (or use a microplate shaker) for 5s and incubate for 45 min at 25°C in the dark.

Step 3: Washing: Uncover the adhesive membrane carefully, discard liquid in the wells, pipette 350µL of **Working Wash Buffer (Solution 6)** to every well, let stand for 30 seconds then drain, repeat 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after tapping dry can be punctured with a clean pipette tip).

Step 4: Color: Add 50µL of **Substrate Reagent A** to each well. Then add 50µL of **Substrate Reagent B** per well. Shake gently by hand (or use a microplate shaker) for 5s,

and allow to react for 15min at 25°C in the dark. (The reaction can be extended appropriately if the blue color is too pale.)

Step 5: Stop the reaction: Pipette 50µL of **Stop Solution** to each well, and shake gently by hand (or use a microplate shaker). The reaction would be stopped.

Step 6: Calculate: Determine the Optical Density (OD value; absorbance value) at 450nm (Reference wavelength 630nm) with a microplate reader. Finish this step within 10min after stop the reaction.

Interpretation of result

◆ Calculate the percentage of absorbance value

$$\text{Percentage of absorbance value(\%)} = \frac{A}{A_0} \times 100\%$$

A—the average OD value of the sample or the standard;

A₀—the average OD value of the 0ppb standard.

It is used to calculate the percentage absorbance of a standard or sample.

◆ Draw the standard curve and calculate

- Take absorbance percentage(A/A₀) of standards as Y-axis and the corresponding log of standards concentration (ppb) as X-axis.
- Draw the standard semi-log curves with X-axis and Y-axis.
- Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve. **Last, the resulting concentration values multiplied by the corresponding dilution times is the actual concentration of SAs of samples.**

If professional analysis software of the kit is used for calculation, it is more convenient for accurate and rapid analysis of a large number of samples.

Attention

- Before test, the reagents and samples should be balanced to room temperature (25°C). If below 25°C, it will lead to all the standard OD value on the low side.
- In washing process, dry wells may result in non-linear standard curves and undesirable reproducibility. Therefore, proceed to the next step immediately after washing.
- Please mix the contents within the wells uniformly and wash the plate thoroughly. The reproducibility is largely determined by consistency of washing step.
- During the incubation, cover microplates with adhesive membrane to avoid light.
- Do not use kits that are overdue. Do not mix reagents with those from other lots.
- Substrate Reagent A/B is colorless. If not, please discard.
- If absorbance value of 0ppb is below 0.5 ($A_{450nm} < 0.5$), it means that the reagent may be metamorphic.
- Stop solution is corrosives. Please avoid contacting with skin.
- **As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.**
- **For the mentioned sample, fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.**
- The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS can be used for quantitative confirmation.