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Cat No.: EKF1086

Sulfonamides (SAs) ELISA Kit

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

Email (Order) order@enkilife.com

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Shelf life: Please refer to the label on the outer package.
Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Product description

This ELISA kit is rapid, quantitative enzyme-linked immunosorbent assays (ELISA) for the determination of Sulfonamides content in Food samples.

Key Features

- Sensitivity：0.5 ppb (ng/mL)
- Detection range：0.5 ppb~40.5 ppb
- Specificity：

Sulfamethazine (SM2)	100%
Sulfamonomethoxine (SMM)	670%
Sulfametoxydiazine (SMD)	582%
Sulfadoxine (SDM')	451%
Sulfamerazine (SM1)	313%
Sulfadiazine (SD/SDZ)	308%
Sulfadimetine (SM2')	241%
Sulfadimethoxine (SDM)	175%
Sulfamethythiadiazole (SMT)	165%
Sulfaguanidine (SG)	131%
Sulfaclozine (Esb3)	67%
Sulfathiazole (ST)	58%
Sulfachloropyridazine (SCPA)	58%
Sulfamethoxypyridazine (SMP)	57%
Sulfaquinoxaline (SQX)	42%
Sulfisoxazole (SIZ)	18%
Sulfamethoxazole (SMZ)	18%

- Detection limits：

Muscle (method 1)	0.5 ppb
Muscle (method 2)	2.5 ppb
Serum, urine	2.0 ppb
Egg, water	1.0 ppb
Honey	0.5 ppb
Milk	10.0 ppb

- Recovery rate：

Muscle, honey, water	95±25%
Serum, urine, milk	85±25%
Egg	90±25%

Storage and Shelf Life

The kit should be stored at 2~8°C and must not be frozen. The shelf life of this product is 1 year. Do not use the kit beyond the expiration date.

Test Principle

This assay employs the competitive inhibition enzyme immunoassay technique. The kit consists of an enzyme-labeled plate pre-coated with coupled antigens, horseradish enzyme markers, antibodies, standard substances, and other supporting reagents. During detection, standard substances or sample solutions are added. Sulfonamides in the samples competes with the pre-coated coupled antigens on the enzyme-labeled plate for Sulfonamides specific antibody. After adding the enzyme markers, TMB substrate is used for color development. The absorbance value of the sample is negatively correlated with the content of Sulfonamides it contains, and the residual amounts of Sulfonamides in the sample can be obtained by comparing with the standard curve.

Materials Supplied

Item	Quantity
Assay Plate	96T
Standard	6 x 1 mL
Concentrated Standard (1 ppm)	1 x 1 mL
HRP Conjugate	1 x 5.5 mL
Antibody	1 x 5.5 mL
TMB Substrate Reagent (A\B)	2 x 6 mL
Stop Solution	1 x 6 mL
Wash Buffer (20×)	1 x 40 mL
Reconstitution Buffer (2×)	1 x 50 mL
Plate Sealer	1 piece
Instruction Manual	1 piece
Sealed Bag	1 copy

Standard Concentration

Standard	1	2	3	4	5	6
Concentration (ppb=ng/mL=ng/g)	0	0.5	1.5	4.5	13.5	40.5

Materials Required, Not Supplied

- Instruments: Microplate reader, printer, homogenizer, nitrogen blow-drying device, vortex, centrifuge, constant temperature incubator, balance (sensitivity 0.01g)
- Single-channel micropipette (20 µL-200 µL,100 µL-1000 µL)

- 300 μL multichannel micropipette
- Reagents: Ethyl acetate, N-hexane, Acetonitrile, $Na_2HPO_4 \cdot 12H_2O$, NaOH, Concentrated HCl, $NaH_2PO_4 \cdot 2H_2O$
- Distilled or deionized water

ⓘNotes:

- Before using the kit, read the instructions carefully.
- Do not use expired kits, and do not mix reagents in kits with different batch numbers.
- 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. Contaminated water or container for reagent preparation will influence the detection result.
- The stop solution is acidic. Wear eyes, hands, face, and clothing protection when using the product.
- EnkiLife is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Reagent Preparation

- **Wash Buffer (1x):** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 10 mL of Wash Buffer (20x) into 190 mL deionized or distilled water to prepare 200 mL of Wash Buffer (1x). Keep it at 4 °C for one month.
- **0.2 M NaOH solution:** Dissolve 0.8 g of **NaOH** to 100 mL with deionized water.
- **0.02 M PB buffer solution:** Dissolve 2.58 g of **$Na_2HPO_4 \cdot 12H_2O$** and 0.44 g of **$NaH_2PO_4 \cdot 2H_2O$** to 500 mL with deionized water.
- **0.5 M HCl solution:** Dissolve 4.3 ml of **Concentrated HCl** to 100 mL with deionized water.
- **Reconstitution Buffer:** Dilute the **Reconstitution Buffer (2×)** with deionized water. (Reconstitution Buffer (2×): Deionized water =1:1). The Reconstitution buffer can be store at 4 ℃ for a month.

Sample Preparation and collection

The prepared sample may be stored for up to one day at 4°C.

Muscle (method 1): Remove fat from sample, homogenize the sample with homogenizer. Add 3±0.05 g of homogeneous muscle sample to a centrifuge tube, then add 3 mL of **0.02 M PB buffer solution**, vortex and mix thoroughly. Add 4 ml of **Ethyl acetate** and 2 mL of **Acetonitrile**, vortex for 10 min, centrifuge at above 4000 rpm for 10 min. Take 2 mL of upper liquid, dry at 50-60℃ with nitrogen evaporators or water bath. Dissolve the residual with 1 mL of **N-hexane**, add 1 mL of **Reconstitution Buffer** and vortex for 1 min. Centrifuge at 4000 rpm for 5 min. Discard the upper N-hexane, take 50 µL of lower liquid for analysis. Dilution factor of sample: 1.

Muscle (method 2): Remove fat from sample, homogenize the sample with homogenizer. Add 2±0.05 g of homogeneous muscle sample to a centrifuge tube, then add 8 mL of **0.02 M PB buffer solution**, vortex for 2 min, centrifuge at above 4000 rpm for 10 min. Take 50 µL of liquid for analysis. Dilution factor of sample: 5.

Serum: Stand blood sample at room temperature for 30 min, centrifuge at 4000 rpm for 10 min, separate the serum. Take 1 mL of serum, add 3 mL of **0.02 M PB buffer solution**, and mix for 30s. Take 50 µL of liquid for analysis. Dilution factor of sample: 4.

Honey: Weigh 1±0.05 g of honey sample into 50 mL centrifuge tube, add 1 mL of **0.5 M HCl solution**, incubate at 37℃ for 30 min. Add 2.5 mL of **0.2 M NaOH solution**, then add 4 mL of **Ethyl acetate**, vortex for 5 min, centrifuge at above 4000 rpm for 10 min at room temperature. Take 2 mL of upper liquid, dry at 50-60℃ with nitrogen evaporators or water bath. (Please do it in a ventilated environment.) Add 0.5 mL of **Reconstitution Buffer** and mix for 30s. Take 50 µL of liquid for analysis. Dilution factor of sample: 1.

Urine: Mix 3 mL of **0.02 M PB buffer solution** and 1 mL of centrifuged clear urine sample fully. Take 50 µL of liquid to analysis. Dilution factor of sample: 4.

Milk: Dilute milk sample with **0.02 M PB buffer solution** with the ratio of 1:19 (for example, 20 µL milk add 380 µL of **0.02 M PB buffer solution**), mix for 30s. Take 50 µL of liquid to analysis. Dilution factor of sample: 20.

Egg: Homogenize the sample with homogenizer. Weigh 2±0.05 g of homogenate egg into a 50 mL centrifuge tube, add 8 mL of **Acetonitrile**. Immediately oscillate for 10 min, centrifuge at 4000 rpm for 5 min at room temperature. Take 2 mL of the supernatant to another 10 mL centrifuge tube (clean and dry), dry at 50-60℃ with nitrogen evaporators or water bath. (Please do it in a ventilated environment.) Dissolve the residue with 1 mL of **N-hexane** and oscillate for 30s. Add 1 mL of **Reconstitution Buffer** and oscillate for 1 min.

Centrifuge at 4000 rpm for 5 min at room temperature. Discard the N-hexane upper layer, and take 50 µL of the lower layer for analysis. Dilution factor of sample: 2.

Water: Take 200 µL of clear water sample (for turbid water sample, centrifuge at 4000rpm for 10 minutes at room temperature to obtain a clear water sample), add 200 µL of **Reconstitution Buffer (2×)**, and mix for 30s. Take 50 µL for analysis. Dilution factor of sample: 2.

Assay Protocol

ⓘNotes:

- Take the required reagent out of the refrigerated environment at 4°C and place it at room temperature for more than 30 min. If the reagent is crystalline, allow it to dissolve sufficiently at room temperature. Shake each liquid reagent well before use.
- It is recommended that all standards and samples be run at least in duplicate.
- Remove the required number of assay plates, put the unused assay plates in sealed bags, and store at 4°C.
- Avoid using metal packaging and stirring reagents.
- Mix the liquid well and complete removal of liquid at each step is essential to good performance.
- Discard the substrate with any color that indicates the degeneration of this solution. When the absorbance value of 0ppb standard less than 0.5 indicates its degeneration.
- The sample addition time for each step shall not exceed 3 min.

1、**Number:** The corresponding microwells of the sample and the standard are numbered in sequence, and each sample and standard are parallel to each well with 2 wells, and the location of the standard well and the sample well is recorded.

2、**Start the reaction:** Add 50 µL of **Standard** or **Sample** per well to the respective microwells, then add 50 µL of **HRP Conjugate** per well, followed by 50 µL of **Antibody** to each well. Cover the assay plate with a plate sealer, gently shake for 5s to mix well, and react in the dark at 25 °C for 45 min.

3、**Washing:** Carefully remove the plate sealer, discard the liquid in the plate wells. Wash by filling each well with 350 µL of **Wash Buffer (1X)** using a squirt bottle, multi-channel pipette, manifold dispenser, or auto washer, and let it stand for 30s. Aspirate or decant the liquid in the plate wells, pat it dry against clean absorbent paper, and complete one wash. Repeating the process 5 times.

Tips: After the plate wells is dried, the next step should be carried out immediately.

4、**Add Substrate Reagent:** Add 50 µL of **Substrate solution A** to each well, then add 50 µL of **Substrate Reagent B**, gently shake for 5s to mix well. Incubate for 15 min at 25°C. Protect from light.

Tips: Adjust the incubation time according to the color change, but do not exceed 30 min. Once the standard wells show a clear gradient, the incubation can be stopped.

5、**Stop the reaction:** Add 50 µL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.

6、**Measure the OD value:** Determine optical density (OD) result at 450 nm within 10 minutes. (Recommend reading the OD value at the dual-wavelength: 450/630 nm).

Calculation of results

1、**Calculation of percent absorbance:**

The percent absorbance of the standard or sample is equal to the average of the percent absorbance value of the standard or sample (double well) divided by the absorbance value of the first standard (0 ppb), multiplied by 100%.

$$\text{percent absorbance (\%)} = \frac{A}{A_0} \times 100\%$$

A—The average absorbance value of the standard or sample solution

A_0—Average absorbance value of 0 ppb standard

2、**Create of standard curve:**

Take the percentage absorbance of the standard substance as the Y-axis and the logarithm of the corresponding standard substance concentration (ppb) as the X-axis to plot the semi-logarithmic curve of the standard substance. The percentage absorbance of the sample is substituted into the standard curve, and the concentration corresponding to the sample is read out from the standard curve, and multiplied by its corresponding dilution factor to get the actual concentration of the analyte in the sample. Substitute the percentage absorbance of the sample into the standard curve, read the concentration corresponding to the sample from the standard curve, and multiply it by the corresponding dilution factor to obtain the actual concentration of the analyte in the sample.

3、**Standard curve reference**

Standard	1	2	3	4	5	6
Concentration (ppb=ng/mL=ng/g)	0	0.5	1.5	4.5	13.5	40.5
OD Standard	2.078	1.628	1.238	0.826	0.417	0.210

ℹ️ We are always committed to providing high-quality products and thank you for your understanding and support. If you have any questions, please feel free to contact our technical support team.