

Sulfamethoxazole (SMZ) ELISA Kit

Technical Manual

(ELISA)



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2.2 Reactive Mode: 25°C, 45min ~ 15min

2.3 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

2.4 Cross-reaction Rate:

Drug name	Cross-reaction Rate	Sensitivity/ppb
Sulfamethoxazole (SMZ)	100%	0.1

2.5 Sample Recovery Rate:

Sample	Recovery rate
Tissue, honey, eggs	85±25%
Urine, milk, serum, feed	80±25%

3 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

4 Materials Required but Not Supplied

4.1 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);

4.2 Micropipette: single-channel (20-200μL and 100-1000μL), and multi-channel 300μL;

4.3 Reagents: Ethyl acetate, n-hexane, acetonitrile, Na₂HPO₄·12H₂O, NaOH, concentrated hydrochloric acid (36% by mass), NaH₂PO₄·2H₂O.

5 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.

5.1 Notice Before Sample Processing:

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

5.2 Solution preparation:

Solution 1: 0.1M Phosphate Buffer

Dissolve 25.8g of Na₂HPO₄·12H₂O and 4.4g of NaH₂PO₄·2H₂O in deionized water to adjust the volume to 1000mL.

Solution 2: Acetonitrile-Ethyl Acetate Solution

Mix 50mL of acetonitrile with 50mL of ethyl acetate in a 100mL glass bottle.

Solution 3: Acetonitrile-Ethyl Acetate Solution

Dissolve 4.3mL of concentrated HCl in deionized water to adjust the volume to 100mL.

Solution 4: 0.2M NaOH Solution

Weigh 0.8g of NaOH, dissolve in deionized water, mix

1 Principle and Application

This kit adopts the method of indirect competitive enzyme-linked immunoassay (ELISA) to detect Sulfamethoxazole (SMZ) in the sample such as tissue, serum, honey, milk 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 SMZ in the samples will compete with the coupled antigens to combine with anti-SMZ antibodies. After adding enzyme conjugates, take coloration with TMB substrates. Absorbance value of the samples is a negative correlation with SMZ content. Lastly, by comparing the obtained absorbance values with the standard curve, we can calculate the SMZ content in the sample.

2 Technique Data

2.1 Kit Sensitivity: 0.1ppb (ng/mL)

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)

5.3 Sample pretreatment steps:

5.3.1 Tissue (higher detection limit) treatment.

1) Weigh 2 ± 0.05 g of homogenized tissue sample into a centrifuge tube. Add 1mL of 0.1M phosphate buffer (Solution 1) and vortex the sample to form a paste. Add 7mL of acetonitrile-ethyl acetate solution (Solution 3) and shake for 2 minutes. Centrifuge at room temperature at 4000r/min or higher for 5 minutes.

2) Transfer 4mL of the clear upper organic phase to a dry container and evaporate to dryness under nitrogen or air at 50-60°C.

3) Dissolve the dried residue in 1mL of n-hexane, add 1mL of reconstitution buffer (Solution 5), and mix by shaking for 30 seconds. Centrifuge at room temperature at 4000r/min or higher for 5 minutes.

4) Remove the upper layer. Take 50μL of the lower layer for analysis.

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

5.3.2 Tissue (lower detection limit) treatment.

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

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

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

5.3.3 Eggs treatment.

1) Homogenize the egg sample using a homogenizer to thoroughly mix the egg white and yolk.

2) Weigh 2.0 ± 0.05 g of the homogenized egg sample (for egg powder, mix 1g with 3mL of deionized water, then take 2mL of this mixture, equivalent to 2g of fresh egg) into a 50mL centrifuge tube. Add 8mL of acetonitrile and immediately shake using a shaker for 10 minutes. Centrifuge at room temperature at 4000r/min or higher for 5 minutes.

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

4) Add 1mL of n-hexane and vortex for 30 seconds to dissolve the dried residue. Then, add 1mL of reconstitution buffer (Solution 5) and vortex for 1 minute. Centrifuge at room temperature at 4000r/min or higher for 5 minutes.

5) Remove the upper layer. Take 50μL of the lower layer for analysis.

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

5.3.4 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 50μL for analysis.

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

5.3.5 Honey treatment.

1) Weigh $1 \text{g} \pm 0.05$ 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) to adjust the pH to around 5, then add 4mL of ethyl acetate, shake for 5 minutes, and centrifuge at 4000r/min at room temperature for 5 minutes.

3) Transfer 2mL of the upper layer to a clean, dry glass

tube and evaporate under nitrogen at 50° C. Add 0.5mL of reconstitution buffer (Solution 5) and vortex for 30 seconds.

4) Take 50μL for analysis.

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

5.3.6 Urine treatment

1) Mix 1 mL of the centrifuged urine sample with 3 mL of 0.1M Phosphate Buffer (Solution 1) and vortex for 30 seconds.

2) Take 50μL for analysis.

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

5.3.7 Milk treatment.

1) Dilute 100μL of milk sample with 0.1M Phosphate Buffer (Solution 1) at a ratio of 1:19 (v/v) (i.e., 100μL of milk with 1.9mL of 0.1M Phosphate Buffer), and vortex for 30 seconds.

2) Take 50μL for analysis.

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

5.3.8 Feed treatment.

1) Weigh $2 \text{g} \pm 0.05$ g of feed sample into a 50mL polystyrene centrifuge tube, add 8mL of acetonitrile, shake for 5 minutes, and centrifuge at 4000r/min at room temperature for 5 minutes.

2) Transfer 1mL of the upper organic phase to a clean, dry 10mL glass tube and evaporate to dryness under nitrogen at 50-60°C.

3) Add 1mL of hexane, vortex for 30 seconds, then add 1mL of 0.1M Phosphate Buffer (Solution 1), vortex for 30 seconds, and transfer to a 2mL polystyrene centrifuge tube. Centrifuge at 4000r/min at room temperature for 5 minutes.

4) Remove the upper organic phase, transfer 100μL of the lower aqueous phase to a 2mL centrifuge tube, add 900μL of 0.1M Phosphate Buffer (Solution 1), and vortex for 1 minute to mix.

5) Take 50μL for analysis

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

6 ELISA procedure I

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.

7 Interpretation of result I

7.1 Calculate the percentage of absorbance value

Percentage of absorbance value(%)= $\frac{A}{A0} \times 100\%$

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

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

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

7.2 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 SMZ 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.

8 Attention I

8.1 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.

8.2 In the washing process, dry wells may result in non-linear standard curves and undesirable reproducibility. Therefore, proceed to the next step immediately after washing.

8.3 Please mix the contents within the wells uniformly and wash the plate thoroughly. The reproducibility is largely determined by consistency of washing step.

8.4 During the incubation, cover microplates with adhesive membrane to avoid light.

8.5 Do not use kits that are overdue. Do not mix reagents with those from other lots.

8.6 Substrate Reagent A/B is colorless. If not, please discard.

8.7 If absorbance value of 0ppb is below 0.5 (A450nm< 0.5), it means that the reagent may be metamorphic.

8.8 Stop solution is corrosives, please avoid contact with skin.

8.9 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.

8.10 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.

8.11 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.

9 Storage conditions I

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.