

SULFAMETHAZINE ELISA TEST KIT
MANUAL

Sulfamethazine (SM2) ELISA Test Kit

Catalogue Number. IP100010

Principle

This test kit is based on the indirect competitive enzyme immunoassay for the detection of Sulfamethazine (SM2) residue. The coupling antigens are pre-coated on the micro-well stripes. The Sulfamethazine in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-Sulfamethazine antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Sulfamethazine in the sample. This value is compared to the standard curve and the Sulfamethazine concentration is subsequently obtained.

Technical specifications

Sensitivity: 1 ppb

Incubator temperature: 25°C

Incubator time: 60min~(20~30)min

Detection limit: Tissue, honey 1 ppb; Serum, urine 4 ppb; Milk 20 ppb

Cross-reaction rate: Sulfamethazine (SM2) 100%

Recovery rate: Tissue 88 ±24%; Milk, honey, serum 79 ±21%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1mL each)	0 ppb, 1 ppb, 3 ppb, 9 ppb, 27 ppb, 81 ppb	
3	Enzyme conjugate	7ml	red cap
4	Antibody working solution	7ml	blue cap
5	Substrate A	7ml	white cap
6	Substrate B	7ml	black cap
7	Stop solution	7ml	yellow cap
8	20× concentrated washing buffer	40ml	white cap
9	2× concentrated redissolving solution	50ml	transparent cap

Materials required but not provided

Equipments: microplate reader, printer, mixer or stomacher, nitrogen-drying device, oscillator, centrifuge, measuring pipets, balance(a reciprocal sensibility of 0.01 g);
 Micropipettors: single-channel 20-200 µL and 100-1000 µL, and multi-channel 30~300 µl;
 Reagents: Acetonitrile(CH₃CN), ethyl acetate, N-hexane, Na₂HPO₄·12H₂O, NaH₂PO₄·2H₂O, NaCl

Sample pre-treatment

Instructions

The following points must be dealt with before the pre-treatment of any kind of sample:

- 1) Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents;
- 2) Before the experiment, each experimental utensil must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution preparation before sample pre-treatment

- 1) 0.2M NaOH solution: Weigh 0.8g NaOH, use 100ml deionized water to dissolve.
- 2) 0.5M HCl solution: 4.3ml concentrated HCl, add deionized water to 100ml, mix evenly.
- 3) 0.02M PB buffer: weight 5.16 g Na₂HPO₄·12H₂O and 0.87 g NaH₂PO₄·2H₂O, dissolve in the deionized water to 1 L.
- 4) The 2× concentrated redissolving solution is mixed with deionized water at 1:1 (1 mL concentrated redissolving solution + 1 mL deionized water), for the sample redissolving.

Samples preparation

a) Tissue

- 1) Weigh 2±0.05 g of the homogenized sample, put into 50ml centrifuge tube, add 6 mL ethyl acetate, mix thoroughly for 2 min, centrifuge at above 4000 r /min at 15 °C for 10 min.
- 2) Transfer 3 mL of the supernatant into a new vessel, blow to dry with nitrogen or air at 50~60 °C.
- 3) Add 1 mL of the diluted redissolving solution, mix evenly, add 1 mL N-hexane, mix for 30s .centrifuge at 4000 r/min at room temperature for 5 min, remove the upper layer.
- 4) Take 20 µL of the lower for further analysis.

Fold of dilution of the sample: 1

b) Serum

- 1) Place sample at room temperature for 30 min, centrifuge at above 4000 r/min at 10 °C for 10 min, separate or filter serum.
- 2) Take 1 mL serum, add 3 mL 0.02 M PB buffer, mix for 30s.
- 3) Take 20 µL for further analysis.

Fold of dilution of the sample: 4

c) Honey

- 1) Put 1.0 ± 0.05 g honey into 50 mL centrifuge tube, add 1 mL 0.5 M HCl, put in 37°C environment for 30 min.
- 2) Add 2.5ml 0.2M NaOH (Adjust PH value to about 5), add 8 mL of the ethyl acetate, shake for 5 min, centrifuge at above 4000 r/min at room temperature (20-25°C) for 10 min.
- 3) Take 4 ml upper layer, blow to dry with nitrogen at 50 °C, add 0.5 mL of diluted redissolving solution, mix for 30s.
- 4) Take 20 µL for further analysis.

Fold of dilution of the sample: 1

d) Urine

- 1) Add 3 mL 0.02 M PB buffer and 1 mL of the centrifuged clear sample, mix properly for 30s.
- 2) Take 20 µL for further analysis.

Fold of dilution of the sample: 4

e) Milk

- 1) Take 1 mL milk, add 0.02 M PB buffer, dilute at 1:19(V/V)
(20 μ L milk + 380 μ L of 0.02 M PB buffer), mix for 30s.
 - 2) Take 20 μ L for further analysis.
- Fold of dilution of the sample: 20*

ELISA procedures

Instructions

- 1) Bring all reagents and micro-well strips to the room temperature (20-25 °C) before use;
- 2) Return all reagents to 2-8 °C immediately after use;
- 3) The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA;
- 4) For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

Operation procedures

Take out the kit from the refrigerated environment.

1. Take out all the necessary reagents and place at the room temperature (20-25 °C) for at least 30min. Note that each reagent must be shaken to mix evenly before use;
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2- 8 °Cn not frozen.
3. Solution preparation: dilute the 20 \times concentrated washing buffer (40 mL) with the deionized water to a final volume of 800 mL (or just to the required volume) for use.
4. Numbering: number the micro-wells according to samples and standard solution; each testing sample and standard solution should be performed in duplicate; record their positions.
5. Add 20 μ L of the sample or standard solution to separate duplicate wells, and add 50 μ L of the enzyme conjugate and then 80 μ L of the antibody working solution into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25 °C for 60min.
6. Wash the microplate with the washing buffer at 250 μ L/well for four to five times. Each time soak the well with the washing buffer for 10 s, flap to dry with absorbent paper (if there are the bubbles after flap ping, cut them with the clean tips).
7. Coloration: add 50 μ L of the substrate A solution and then 50 μ L of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 °C for 20~30 min at dark for coloration.
8. Determination: add 50 μ L of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (we recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min)

Result judgment

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the Sulfamethazine concentration.

Qualitative determination

The concentration range (ng/mL) obtained from the comparison the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample I is 0.3, and that of the sample

II is 1.0, the OD value of standard solutions is: 2.243 for 0ppb, 1.816 for 1ppb, 1.415 for 3ppb, 0.74 for 9ppb, 0.313 for 27ppb, 0.155 for 81ppb, accordingly the concentration range of the sample I is 27ppb to 81ppb, and that of the sample II is 3ppb to 9ppb. (multiplied by the corresponding dilution fold)

Quantitative determination

The mean values of the absorbance values obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B_0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

$$\text{Percentage of absorbance value} = (B / B_0) \times 100\%$$

B—the average OD value of the sample or the standard solution

B_0 —the average OD value of the 0 ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithm values of the Sulfamethazine standard solutions (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the Sulfamethazine concentration in the sample.

Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software)

The individual Sulfonamides result calculation method

Results multiply the cross-reactivity rate

Precautions

1. The room temperature below 25 °C or the temperature of the reagents and the samples being not returned to the room temperature (20-25 °C) will lead to a lower standard OD value.
2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.
3. Mix evenly, otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.
6. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.
7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solution of less than 0.5 ($A_{450\text{ nm}} < 0.5$) indicates its degeneration.
8. The optimum reaction temperature is 25°C, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

Storage: store at 2-8 °C, not frozen.

Expiry date: 12 months; date of production is on box.