VERSION 1.01

SULFAMETHOXAZOLE ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Sulfamethoxazole ELISA Test Kit

Catalogue Number. IP100013

Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Sulfamethoxazole(SMZ) residue. The coupling antigens are pre-coated on the micro-well stripes. The Sulfamethoxazole(SMZ) in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-Sulfamethoxazole(SMZ) 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 Sulfamethoxazole(SMZ) in the sample. This value is compared to the standard curve and the Sulfamethoxazole(SMZ) concentration is subsequently obtained.

Technical specifications

Sensitivity: 1 ppb
Detection limit: Meat, liver, pork, chicken, honey, egg1 ppb; Serum, urine4 ppb; Milk20 ppb
Recovery rate: Meat, chicken, pork, liver70 ±10%, Egg65 ±10%, Milk ,honey,serum70 ±10%
Cross-reaction rate: Sulfamethoxazole (SMZ)100%, Sulfamerazine (SM_1)12%

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 and 81 ppb	
3	Enzyme conjugate	7ml	red cap
4	Antibody working solution	10ml	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, homogenizer, nitrogen-drying device, votex, centrifuge, measuring pipets, balance (a sensibility reciprocal of 0.01 g), Incubator
Micropipettors: single-channel 20-200 µL and 100-1000 µL, and multi-channel 250 µL
Reagents: Acetonitrile (CH3CN), ethyl acetate, N-hexane, Na2HPO4·12H2O, NaH2PO4·2H2O, NaOH, HCl

Sample pre-treatment

Instructions

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) 0.2 M NaOH: dissolve 0.8 g NaOH in deionized water to 100 mL.
2) 0.5 M HCl (for honey): dissolve 4.3 mL HCl (36%) in deionized water to 100 mL.
3) 0.02 M PB buffer: dissolve 2.58 g $Na_2HPO_4 \cdot 12H_2O$ and 0.44 g $NaH_2PO_4 \cdot 2H_2O$ in the deionized water to 500 mL. (for high-detection-limit samples)
4) The 2× concentrated redissolving solution is diluted with deionized water at 1:1 (1 mL concentrated red issolbing solution + 1 mL deionized water), used for the treated sample redissolving

Samples preparation

High-detection-limit samples

a) Animal tissues , meat , liver, shrimp, fish and egg
1. Homogenize the sample at 10000 r/min for 1 min
2. Weigh 3± 0.05 g of the homogenized sample, put into centrifugal tube; Add 3 mL 0.02M PB buffer, then add 4 mL ethyl acetate and 2 mL Acetonitrile (CH3CN), shake properly for 10 min, centrifuge at above 4000 r/min at 15 ℃ for 10 min;
3. Transfer 2 mL supernatant into a new centrifuge tube,blow to dry completely with nitrogen or air at 50-60 ℃;
4. Add 1 mL N-hexane to dissolve the dry residue, then add 1 Ml of the diluted redissolving solution, shake the dry residue, then add 1 mL of the diluted solution, shake strongly for 1 min, centrifuge at 4000 r/min at room temperature for 5 min, remove the upper layer;
5. Take 20 µL underlayer for further analysis.
Fold of dilution of the sample: one

<u>It needs five fold to dilute the sample(1mL sample+4 mL of the diluted redissolving solution) if the detecti on is stipulated in the most residue (100 ppb) of national regulation.</u>

Low-detection-limit samples

a) Animal tissues (meat, liver and so on)

1. Weigh 2.0 ± 0.05 g of the sample, add 10 mL 0.02 M PB buffer, shake upside down for 10 min, put into constant temperature container at 37 ℃ for 30 min, then centrifuge at above 5000 r/min at room temprature (20-25 ℃) for 10 min;
2. Take 20 µL of the clear supernatant (upper layer) for further analysis.
Fold of dilution of the sample: 5
Detection limt: 5 ppb

b) Animal tissues (chicken, liver)

1. Weigh 2.0 ± 0.05 g of the sample, add 10 mL 0.02 M PB buffer and 5 mL N hexane, shake upside down for 10 min, centrifuge at above 5000 r/min at room temprature (20-25 ℃) for 10 min.
2. Remove N-hexane of upper layer, take 100 µL of the lower, add 100 µL 0.02 M PB buffer, mix properly.
3. Take 20 µL for analysis.
Fold of dilution of the sample: 10
Detection limt: 10 ppb

c) Serum

1. Place blood sample at room temperature (20-25 ℃) for 30 min, centrifuge at above 4000 r/min at room temperature(20-25 ℃) for 10 min, separate or filter serum
2. Take 1 mL serum, add 3 mL 0.02 M PB buffer, mix properly
3. Take 20 µL for further analysis

Fold of dilution of the sample: 4

Detection limt: 4ppb

d) Honey

1. Put 1.0 ± 0.05 g honey into 50 mL centrifugal tube, add 1 mL 0.5 M HCl, at 37 ℃, for 30 min.
2. Add 2.5 mL 0.2 M NaOH (pH is approx 5), add 4 mL ethyl acetate, shake for 10 min, centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min.
3. Take 2 mL supernatant, blow to dry with nitrogen at 50 ℃, add 0.5 mL of the diluted redissolving solution, redissolve it.
4. Take 20 µL for further analysis

Fold of dilution of the sample: 1

e) Urine

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

Fold of dilution of the sample: 4

Detection limt: 4 ppb

f) Milk

1. Take 1 mL milk, add 0.02 M PB buffer, dilute at 1:19(V / V) (20 µL milk + 380 µL 0.02 M PB buffer)
2. Take 20 µL for further analysis

Fold of dilution of the sample: 20

Detection limt: 20 ppb

ELISA procedures

Instructions

1. Bring all reagents and micro-well strips to the room temperature (20-25 ℃) before use;
2. Return all reagents to 2-8 ℃ 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

1. Take out all the necessary reagents and place at the room temperature (20-25 ℃) 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 ℃;

3. Solution preparation: dilute the 20× concentrated washing buffer with the distilled or deionized water to 800 mL (or just to the required volume) for use;
4. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions;
5. Add 20 µL of the sample or standard solution to separate duplicate wells, add 50 µL of enzyme conjugate then add 80 µL of the antibody then add working solution into each well.
Mix by shaking gently, seal the microplate with the cover membrane, and incubate at 25℃ for 1h;
6. Wash the microplate with the washing buffer at 250 µL/well for 4-5 time; soak the well with the washing buffer for 15-30 sec, flap to dry with absorbent paper.
(if there are the bubbles after flapping, cut them with the clean tips);
7. Coloration: add 50 µL of the substrate A solution and 50 µL of the B solution into each well. Mix by shaking gently, and incubate at 25 ℃ for 20-30 min in the dark for coloration;
8. Determination: add 50 µL of stop solution into each well. Mix by shaking gently. Set the wavelength of the microplate reader at 450 nm to determine the OD value. (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 con tent of Sulfonamides in the sample.

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 0.211, and that of the sample Ⅱ is 0.785, the OD value of standard solutions is: 2.140 for 0ppb, 1.560 for 1ppb, 1.124 for 3ppb, 0.650 for 9ppb, 0. 328 for 27ppb, 0.125 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 percentage of the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

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 Sulfamethoxazole(SMZ) 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 Sulfamethoxazole(SMZ) 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)

Precautions

1. The room temperature below 25 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) 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 (A450 nm< 0.5) indicates its degeneration.
8. The optimum reaction temperature is 25℃, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

Storage: store at 2-8 ℃, not frozen.
Expiry date: 12 months; date of production is on box.