VERSION 1.01

SULFAMETHOXYDIAZINE ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Sulfamethoxydiazine ELISA Test Kit

Catalogue Number. IP100011

Principle

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

Technical specifications

Sensitivity: 0.4ppb
Incubator temperature: 25℃
Incubator time: 40min～20min
Detection limit: Tissue (high-detection-limit method) 0.4 ppb, Tissue (lower-detection-limit method) 2 ppb, Honey 0.4 ppb, Serum, urine 1.6 ppb, Milk 8 ppb
Cross-reaction rate: Sulfamethoxydiazine (SMD) 100%
Recovery rate: Urine,Milk,serum 70 ±23%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1mL each)	0 ppb, 0.4 ppb, 1.2 ppb, 3.6 ppb, 10.8 ppb, 32.4 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, homogenizer, nitrogen-drying device, votex, centrifuge, measuring pipets, balance (a sensibility reciprocal of 0.01 g)
Micropipettors: single-channel 20-200 µL, 100-1000 µL, and multi-channel 30-300 µL;
Reagents: Acetonitrile (CH_3CN), ethyl acetate, N-hexane, $Na_2HPO_4{\cdot}12H_2O$, $NaH_2PO_4{\cdot}2H_2O$, NaOH, HCl, Methylene chloride

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) 0.2 M NaOH: dissolve 0.8 g NaOH in deionized water to 100 mL.
2) 0.5 M HCl: dissolve 4.3 mL HCl (36%) in deionized water to 100 mL.
3) 0.02 M PB buffer: dissolve 5.16g $Na_2HPO_4 \cdot 12H_2O$ and 0.87g $NaH_2PO_4 \cdot 2H_2O$ in the deionized water to 1L.
4) Acetonitrile (CH_3CN) -Methylene chloride mixing solution
$V_{acetonitrile}$-$V_{methylene\ chloride}$ = 1 : 4
5) The 2× concentrated redissolving solution is diluted with deionized water at 1:1 (1 mL concentrated redissolbing solution + 1 mL deionized water), used for the treated sample redissolving.

Samples preparation

a) Tissues (chicken, duck, pork/liver, egg, fish, shrimp etc.)

A. High-detection-limit method
Method one

1) Weigh 2.0 ± 0.05 g of the homogenized tissue sample into 50 ml centrifuge tube
2) Add Acetonitrile (CH_3CN) -Methylene chloride mixing solution 8 ml, shake for 2 min, centrifuge at above 4000 r/min at 15 ℃ for 10 min
3) Take 4 ml the clear organic phase (upper layer) into a dry tube, blow to dry with nitrogen or air completely by rotary evaporation at 50-60 ℃
4) Dissolve the dry residues in 1 mL of the diluted redissolving solution, add 1 mL N-hexane, shake vigorously for 30 seconds; centrifuge at above 4000 r/min at 15℃ for 5 min.
5) Remove the upper layer, take 20µl lower layer solution for further analysis.
Fold of dilution of the sample: 1

Method two

1) Weigh 3 ± 0.05 g of the homogenized sample, put into centrifugal tube, add 3 mL 0.02 M PB buffer, shake properly. Then add 4 mL ethyl acetate and 2 mL Acetonitrile (CH_3CN), shake properly for 10 min, centrifuge at above 4000 r/min at 15 ℃ for 10 min;
2) Transfer 2 mL supernatant(approx 1 g sample) into a new centrifugal tube, blow to dry with nitrogen or air completely by rotary evaporation at 50-60 ℃
3) Add 1 mL N-hexane, then add 1 mL of the diluted redissolving solution, shake strongly for 30 s. Centrifuge at 4000 r/min at room temperature for 5 min, remove the upper layer
4) Take 20 µL lower layer solution for further analysis.
Fold of dilution of the sample: 1

B. Tissue lower-detection-limit method

1) Weigh 2.0 ± 0.05 g of the homogenized sample into a 50 ml centrifugal tube, add 8 mL 0.02 M PB buffer, shake for 2 min, centrifuge at above 4000 r/min at 15 ℃ for 10 min;

2) Take 20 µL for further analysis.
Fold of dilution of the sample: 5

b) Serum

1) Place the serum sample in the room temperature for 30 min, centrifuge at above 4000r/min at 10 ℃ for 10 min, separation of the serum or filter serum
2) Take 1 mL serum and add 3mL 0.02 M PB buffer. Mix for 30 s.
3) Take 20 µL for further analysis
Fold of dilution of the sample: 4

c) Honey

1. Weight 1.0 ± 0.05 g honey into 50 mL centrifugal tube, then add 1 mL 0.5 M HCI. Be static at 37 ℃ for 30 min.
2. Add 2.5 mL 0.2 M NaOH (adjust pH to 5), then add 4 mL ethyl acetate, shake for 5 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 for 30 s.
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.
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 µL milk + 380 µL 0.02 M PB buffer), mix for 30s.
2. Take 20 µL 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℃);
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 the kit from the refrigerated environment. Take out all the necessary reagents from the kit and place at the room temperature (20-25℃) for at least 30 min. 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℃, not frozen.
3. Solution preparation: dilute 40 mL of the 20× concentrated washing buffer with the 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, and add 50 µL of the enzyme conjugate, and then 80 µL 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℃ for 40min.
6. Wash the microplate with the washing buffer at 250 µL/well for 4-5 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 flapping, cut them with the clean tips).
7. Coloration: add 50 µL of the substrate A solution and then 50 µL of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃ for 20 min at dark for coloration;
8. Determination: add 50 µL 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 Sulfamethoxydiazine concentration.

Qualitative determination

The concentration range (ng/mL) can be obtained from comparing the average OD value of the samplewith 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 0.4ppb, 1.415 for 1.2ppb, 0.74 for 3.6ppb, 0.313 for 10.8ppb, 0.155 for 32.4ppb, accordingly the concentration range of the sample Ⅰ is 10.8ppb to 32.4ppb, and that of the sample Ⅱ is 1.2ppb to 3.6ppb. (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,

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 Sulfamethoxydiazine 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 actual Sulfamethoxydiazine 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 testing 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 (A_{450nm}< 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.