VERSION 1.01

SULFAQUINOXALINE ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Sulfaquinoxaline ELISA Test Kit

Catalogue Number. IP100012

Principle

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

Technical specifications

Sensitivity: 1 ppb
Incubator temperature: 25℃
Incubator time: 60min～(20~30)min
Detection limit: Tissue, honey 1 ppb; Serum, urine 4 ppb; Milk 20 ppb
Cross-reaction rate: Sulfaquinoxaline (SQX) 100%
Recovery rate: Tissue 90 ±22%, Milk, honey, serum 83 ±17%

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, 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: Ethyl acetate, N-hexane, $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$, NaOH, HCl

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, dissolve with 100ml deionized water;
2) 0.5M HCl solution: dissolve 4.3ml HCL with deionized water to 100ml.
3) 0.02 M PB buffer: weigh 5.16 g $Na_2HPO_4 \cdot 12H_2O$ and 0.87 g $NaH_2PO_4 \cdot 2H_2O$, dissolve with 1L deionized water.
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) Animal tissues (meat, liver) egg, fish, shrimp etc.

1) Weigh 2±0.05 g tissue sample into 50ml centrifuge tube, add 6 mL ethyl acetate, mix evenly for 2 min, centrifuge at above 4000 r /min at 15 ℃ for 10 min.
2) Transfer 3 mL of the clear liquid (upper layer), blow to dry with nitrogen or air at 50-60 ℃.
3) Add 1 mL diluted redissolving solution, then add 1 mL N-hexane to dissolve the dry residue, shake vigorously for 30s, centrifuge at 4000 r/min at room temperature(20-25 ℃) for 5 min, remove the upper layer of liqiud.
4) Take 20 µL of the lower for further analysis.
Fold of dilution of the sample: 1

b) Serum

1) Place serum at room temperature (20-25℃) for 30 min, centrifuge at above 4000 r/min at 10 ℃ for 10 min, separate or filter serum.
2) Take 1 mL serum, add 3 mL 0.02 M PB buffer, mix properly 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 0.5M HCl, put at 37℃ environment for 30min..
2) Add 2.5ml 0.2M NaOH solution (adjust PH value to 5), then add 8 mL ethyl acetate, shake for 5 min, centrifuge at above 4000 r/min at room temperature (20-25℃) for 10 min.
3) Take 4 ml upper layer liquid, blow to dry with nitrogen at 50 ℃, add 0.5 mL diluted redissolving solution to redissolve, mix for 30s.
4) Take 20 µL for further analysis.
Fold of dilution of the sample: 1

d) Urine

1) Add 3 mL of 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 µL milk + 380 µL of 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 temperature, 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 from the kit and place at the room temperature (20-25 ℃) for at least 30 min. Note that each liquid 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 the 20× concentrated washing buffer (40 mL) with deionized water to a final volume of 800 mL 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 sample or standard solution to separate duplicate wells, and add 50 µL enzyme conjugate and then 80 µL antibody working solution into each well. Vortex evenly, seal the microplate with the cover membrane, and incubate at 25 ℃ for 1 h.
6. Wash the microplate with the washing buffer at 250 µL/well for 4-5 times. Each time soak the wells 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 substrate A solution and then 50 µL B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃ for 20-30 min at dark for coloration..
8. Determination: add 50 µL 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 sulfaquinoxaline concentration.

Qualitative determination

The concentration range (ng/mL) can be obtained from comparing 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 0 ppb, 1.816 for 1ppb, 1.415 for 3 ppb,

0.74 for 9 ppb, 0.313 for 27 ppb, 0.155 for 81 ppb, accordingly the concentration range of the sample Ⅰ is 27 to 81 ppb, and that of the sample Ⅱ is 3 to 9 ppb.

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 Sulfaquinoxaline 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, thus finally obtaining the Sulfaquinoxaline 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 reagents and 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.