VERSION 1.01

SULFADIAZINE ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Sulfadiazine ELISA Test Kit

Catalogue Number. IP100014

Principle

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

Technical specifications

Sensitivity: 1 ppb
Incubator temperature: 25℃
Incubator time: 30min～15min
Detection limit
Tissue (high-detection-limit method) 1 ppb; Tissue (lower-detection-limit method) 5 ppb; Honey 1 ppb
Serum, urine 4 ppb; Milk 20 ppb
Cross-reaction rate: Sulfadiazine (SD or SDZ) 100.0%; Sulfamonomethoxine (SMM) 7%; Sulfamerazine (SM_1) 12%; Sulfamethoxydiazine(SMD) 12.5%
Recovery rate: Tissue, urine, milk 85±25%; Honey, serum 80±23%

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	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	20×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, 100-1000 µL, and multi-channel 30-300 µL;
Reagents: Acetonitrile (CH_3CN), ethyl acetate, N-hexane, $K_2HPO_4{\cdot}12H_2O$, Citric acid monohydrate ($C_6H_8O_7{\cdot}H_2O$), HCl, NaOH, CH_2Cl_2,

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) 0.2M NaOH solution: Weigh 0.8g NaOH, dissolve with 100ml deionized water;
2) 0.5M HCl: Take 4.3ml HCl, dissolve with deionized water to 100ml, mix evenly;
3) Na_2HPO_4- $C_6H_8O_7 \cdot H_2O$ buffer: weigh 19.85g$Na_2HPO_4 \cdot 12H_2O$ and 9.3g $C_6H_8O_7 \cdot H_2O$, dissolve with deionized water to 1L, mix evenly;
4) CH_3CN-CH_2Cl_2 solution: V_{CH3CN}: V_{CH2Cl2} =1:4 ;
5) The 20× concentrated redissolving solution is diluted with deionized water at 1:19(1 part concentrated redissolbing solution + 19 parts deionized water).

Samples preparation

a) Tissue
High-detection-limit method

Method one

1) Weigh 2.0 ± 0.05 g of the homogenized tissue sample into 50 ml centrifuge tube, add 6 ml ethyl acetate, shake for 2 min, centrifuge at above 4000 r/min at 15 ℃ for 10 min;
2) Take 3 ml the clear organic phase into a dry container, blow to dry with nitrogen or air completely by rotary evaporation at 50-60 ℃
3) Dissolve the dry residues in 1 mL of the diluted redissolving solution, add 1 mL N-hexane, mix for 30 seconds; centrifuge at above 4000 r/min at 15℃ for 5 min. Remove the upper layer N-hexane phase,
4) Take down-layer 50µl solution for further analysis.
Fold of dilution of the sample: 1

Method two

1) Weigh 2 ± 0.05 g of the homogenized sample, put into 50ml centrifugal tube;
2) Add 8ml CH_3CN-CH_2Cl_2 solution, shake for 5min, centrifuge at above 4000 r/min at 15 ℃ for 10 min;
3) Transfer 4 ml organic phase into a dry container, blow to dry with nitrogen or air completely by rotary evaporation at 56 ℃
4) Add 1 mL of the diluted redissolving solution to redissolve the dry residue, add 1 mL N-hexane, shake for 30s. Centrifuge at above 4000 r/min at 15℃ for 5 min;
5) Remove the upper layer N-hexane phase. Take 50 µL down layer solution for further analysis.
Fold of dilution of the sample: 1

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 diluted redissolving solution, shake for 2 min, centrifuge at above 4000 r/min at 15 ℃ for 10 min;
2) Take 50 µL solution 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 the diluted redissolving solution, mix for 30s.
3) Take 50 µL solution for further analysis
Fold of dilution of the sample: 1

c) Honey

1. Weight 1 ± 0.05 g honey sample into 50 mL centrifugal tube, add 1ml 0.5M HCl solution, put it into 37℃ environment for 30min;
2. Add 2.5ml 0.2M NaOH solution and 3ml Na_2HPO_4- $C_6H_8O_7 \cdot H_2O$ buffer separately, then add 4ml ethyl acetate, shake for 2min, centrifuge at above 4000 r/min at 15 ℃ for 10 min;
3. Take 2mL up-layer organic phase, blow to dry with nitrogen at 50-60 ℃, add 0.5 mL of the diluted redissolving solution to redissolve, mix for 30s.
4. Take 50 µL for further analysis
Fold of dilution of the sample: 1

d) Urine

1. Add 3 mL the diluted redissolving solution and 1 mL of the centrifuged clear urine sample, mix properly for 30s.
2. Take 50 µL for further analysis
Fold of dilution of the sample: 4

e) Milk

1. Take 1 mL milk, add the diluted redissolving solution, dilute at 1:19(V / V) (20 µL milk + 380 µL the diluted redissolving solution), mix for 30s.
2. Take 50 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 ℃) 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. 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.

6.2 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. Washing buffer preparation: dilute 40 mL of the 20× concentrated washing buffer with the deionized water at 1:19 (1 part 20× concentrated washing buffer + 19 parts deionized water). Or prepare washing buffer as quantity needed.
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 50 µL of the sample or standard solution to separate duplicate wells, add 50 µL of enzyme conjugate, then add 50 µL of the antibody working solution into each well. Mix by shaking gently, seal the microplate with the cover membrane, and incubate at 25℃ for 30 min;
6. Wash the microplate with the washing buffer at 250 µL/well for four to five times.;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 15 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 content of Sulfadiazine 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 0.3, 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 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 Sulfadiazine 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 Sulfadiazine 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.