Datasheet for ABIN400607

Sulfamethoxydiazine ELISA Kit

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Overview

Quantity:	96 tests
Target:	Sulfamethoxydiazine
Reactivity:	Chemical
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Purpose:	This test kit is based on the competitive enzyme immunoassay for the detection of Sulfamethoxy- diazine 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 samplehas 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.
Analytical Method:	Qualitative and Quantitative
Detection Method:	Colorimetric
Components:	Micro-well strips: 12 strips with 8 removable wells each 6 standard solution (1 mL each): 0 ppb, 1 ppb, 3 ppb, 9 ppb, 27 ppb and 81 ppb, Enzyme conjugate (7 mL) red cap, Antibody working solution (10 mL) blue cap, Substrate A solution (7 mL) white cap, Substrate B solution (7 mL) black cap, Stop solution (7 mL) yellow cap, 20 concentrated washing buffer (40 mL) white cap, 2 concentrated redissolving solution (50 mL) transparent cap

Product Details

Material not included:	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 L and 100-1000 L, and multi-channel 250 L, 3) Reagents: Acetonitrile (CH3CN), ethyl acetate, N-hexane, Na2HPO412H2O, NaH2PO42H2O, NaCl

Target Details

Target:	Sulfamethoxydiazine
Abstract:	Sulfamethoxydiazine Products
Target Type:	Chemical

Application Details

Plate:	Pre-coated
Protocol:	**Sample pre-treatment:** Instructions (The following points must be dealt with before the pre-treatment) Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents, 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 2 M NaCl: dissolve 11.69 g NaCl in deionized water to 100 mL. 2 0.02 M PB buffer: weight 2.58 g Na2HPO412H2O and 0.44 g NaH2PO42H2O, dissolve in the deionized water to 500 mL. 3 Acetonitrile (CH3CN)-water solution: ?CH3CN / ?H2O =84:16 4 The 2 concentrated redissolving solution is mixed with deionized water at 1:1 (1 mL 2 concentrated redissolving solution+1 mL deionized water), for the sample redissolving. 5.1 High-detection-limit samples 5.1.1 Animal tissues (meat, liver), shrimp, fish, egg. Take the sample, homogenize at 10000 r/min for 1 min. Weigh 30.05 g of the homogenized sample, put into centrifugal tube, add 9 mL of the CH3CN-water solution, shake properly for 10 min, centrifuge at above 4000 r /min at 15 ? for 10 min. Transfer 4 mL of the supernatant into a new vessel, add 2 mL 2 M NaCl solution and 7 mL of ethyl acetate, shake for 10 min, and centrifuge at above 4000 r/min at 15? for 5 min. Transfer the supernatant into a new vessel, blow to dry with nitrogen completely by rotary evaporator at 50oC. Add 1 mL of the diluted redissolving solution, shake for 1 min, add 1 mL n-hexane, mix for 2 min and centrifuge at 4000 r/min at 15? for 5 min, remove the liqiud (upper layer). Take 20 L of the lower for further analysis. Fold of dilution of the sample: 1 It needs five fold dilution of the sample(1 mL sample+4 mL of the diluted redissolving solution) if the detection is based on the most residue (100 ppb) of national regulation. 5.2 Low-detection-limit samples 5.2.1 Animal tissues (meat, liver) 1) Take 2.0 0.05 g of the sample, add 10 mL

0.02 M PB buffer, shake upside down for 10 min, put into 37? constant temperature container for 30 min, centrifuge at above 5000 r/min at 10? for 10 min. 2) Take 20 L of the clear supernatant (upper layer) for further analysis. Fold of dilution of the sample: 5 detect limit: 5 ppb 5.2.2 Animal tissues (chicken, liver) 1) Take 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 10oC for 10 min. 2) Remove n-hexane phase (upper layer), take 100 L of the lower, add 100 L 0.02 M PB buffer, mix vigorously. 3) Take 20 L for analysis Fold of dilution of the sample: 10 detect limit :10 ppb 5.2.3 Serum 1) Place sample at room temperature for 30 seconds, centrifuge at above 4000 r/min at 10oC for 10 min, separate or filter serum. 2) Take 1 mL serum, add 3 mL 0.02M PB buffer, mix properly. 3) Take 20 L for further analysis. Fold of dilution of the sample: 4 detect limit :4 ppb 5.2.4 Honey 1) Put 1.0 0.05 g honey into 50 mL centrifugal tube, add 2 mL 0.02 M PB buffer, shake properly. 2) Add 8 mL ethyl acetate, shake for 10 min, centrifuge at above 4000 r/min at room temperature (20-25?) for 10 min. 3) Take 4 mL supernatant (upper layer), blow to dry with nitrogen at 50oC, add 0.5 mL of the diluted redissolving solution to redissolve. 4) Take 20 L for further analysis Fold of dilution of the sample: 1 5.2.5 Urine 1) And 3 mL 0.02 M PB buffer and 1 mL of the centrifuged clear sample, mix properly. 2) Take 20 L for further analysis Fold of dilution of the sample: 4 detect limit :4 ppb 5.2.6 Milk Take 1 mL milk, add 0.02 M PB buffer, dilute at 1:20(?/?) (20 L milk + 380 L 0.02 M PB buffer) Take 20 L for further analysis Fold of dilution of the sample:20 detect limit :20 ppb

ELISA procedures Instructions: Bring all reagents and micro-well strips to the room temperature (20-25?), Return all reagents to 2-8oC immediately after use, 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, 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. Operating 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 2-8oC, not frozen. Solution preparation: dilute 40 mL of the 20 concentrated washing buffer with the distilled or deionized water to 800 mL (or just to the required volume) for use. Numbering: number the micro-wells according to samples and standard solution, each sample and standard solution should be performed in duplicate, record their positions. Add 20 L of the sample or standard solution to separate duplicate wells, and add 50 L of the enzyme conjugate, and then 80 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? for 1 h. Wash the microplate with the washing buffer at 250 L/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 L of the substrate A solution and then 50 L of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 25oC for 20-30 min at dark for coloration (See precaution 8), 8 Determination: add 50 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).

Interpretation of results 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 samplehas a negative correlation with Sulfamethoxydiazine concentration. 7.1 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 1 is 0.211, and that of the sample 2 is 0.785, the OD value of standard solutions is: 2.140 for 0 ppb, 1.560 for 1 ppb, 1.124 for 3 ppb, 0.650 for 9 ppb, 0.328 for 27 ppb ,0.125 for 81 ppb, accordingly the concentration range of the sample 1 is 27 to 81 ppb, and that of the sample 2 is 3 to 9 ppb. (multiplied by the corresponding dilution fold). Quantitative determination: The mean values of the absorbance values is equivalent to the percentage of the average OD value (B) of the sampleand 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 100% B0 Bthe average (double wells) OD value of the sample or the standard solution B0the average OD value of the 0ng/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** The room temperature below 20oC or the temperature of the reagents and the testing samples being not returned to the room temperature (20-25oC) will lead to a lower standard OD value. 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. Mix evenly, otherwise there will be the undesirable reproducibility. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin. 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

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	sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use. 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. 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 (A450nm< 0.5) indicates its degeneration.
Restrictions:	For Research Use only

Handling

Storage:	4 °C