Sulfadimethoxine ELISA Kit

Prod. No.: DEIA034
Pkg. Size: 96T

1. Background

Sulfadimethoxine (SDM) belongs to the sulfonamides family, which are very important in controlling and curing animal disease. These drugs have very serious side effects and will lead to SAs resistance of some bacilus if they exist in human body for a long period. They also have potential carcinogenecity. Strict MRLs have been set for SAs in EU, US and Japan.

This kit is a new generation product for drug residue detection based on ELISA technology. It is fast, simple, accurate and sensitive. And it requires only 1.5 hours in one operation, which considerably minimizes work intensity and operation error.

2. Test Principle

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. SDM in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme conjugate, TMB substrate is used to show the color. Absorbance of the sample is negatively related to the SDM residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, SDM residue quantity in the sample can be calculated.

3. Applications

This kit can be used in quantitative and qualitative analysis of SDM residue in animal tissue (muscle, liver, fish and shrimp), egg, honey, milk, urine and serum.

4. Cross-reactions

Sulfadimethoxine……..……..……100%
Other sulfa drugs……..……..……1%

5. Materials Required

5.1 Equipments:

----Microtiter plate spectrophotometer (450nm/630nm)
----Rotary evaporator or nitrogen drying instruments
----Homogenizer
----Shaker
----Vortex Mixer
----Centrifuge
----Analytical balance (inductance: 0.01g)
----Graduated pipette: 10ml
----Rubber pipette bulb
----Volumetric flask: 100ml, 500ml, 1L
----Glass test rube: 10ml
----Polystyrene Centrifuge tube: 2ml, 50ml
----Micropipettes: 20μl-200μl, 100μl-1000μl, 250μl -multiple

5.2 Reagents

-- Acetonitrile (AR)
-- Ethyl acetate (AR)
-- N-hexane (AR)
-- Sodium chloride (NaCl, AR)
-- Disodium hydrogen phosphate 12-hydrate ($Na_2HPO_4.12H_2O$, AR)
-- Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4.2H_2O$, AR)
-- Deionized water

6. Kit Components

– Microtiter plate with 96 wells coated with antigen
– Standard solutions(6 bottles×1ml/bottle)
0ppb, 1ppb, 3ppb, 9ppb, 27ppb, 81ppb
– Spiking standard solution: (1ml/bottle) **1ppm**
– Enzyme conjugate 7ml……. ……..…red cap
– Antibody solution 7ml……...……..green cap
– Solution A 7ml ……..…….....white cap
– Solution B 7ml ……..…….....red cap
– Stop solution 7ml….....….yellow cap
– 20×Concentrated wash solution 40ml .....……transparent cap
– 20×Concentrated extraction solution 50ml….blue cap

7. Reagent preparation:

Solution 1: 2M NaCl

Dissolve 11.69g of NaCl with water and dilute to 100ml.

Solution 2: 0.02M PBS

Dissolve 2.58g of $Na_2HPO_4.12H_2O$, 0.44g of $NaH_2PO_4.2H_2O$ with deionized water and dilute to 500ml.

Solution 3: Acetonitrile solution (for animal tissue, egg)

Mix 84ml of anhydrous acetonitrile with 16ml of deionized water.

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Solution 4: Extraction solution

Dilute the 20×concentrated extraction solution with deionized water in the volume ration of 1:19(*e.g. 10ml of 20×concentrated extraction solution + 190ml of deionized water*), which will be used for sample extraction. This diluted solution can be conserved for 1 month at 4℃.

Solution 5: wash solution

Dilute the 20×concentrated wash solution with deionized water in the volume ration of 1:19(*e.g. 10ml of 20×concentrated wash solution + 190ml of deionized water*), which will be used to wash the plates. This diluted solution can be conserved for 1 month at 4℃.

8. Sample Preparations

8.1 Notice and precautions before operation:

(a) Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.

(b) Make sure that all experimental instruments are clean.

(c) Samples must be used for assay after diluted with extraction solution.

8.2 Animal tissue

8.2.1 Method A (high detection limit)

(chicken/liver, pork/liver, eggs, fish , shrimp)

----Homogenize the sample with homogenizer.

Note: *for egg sample, mix the egg white and yolk completely.*

----Weigh 3.0±0.05g of homogenate to a 50ml polystyrene centrifuge tube, add 9ml of acetonitrile solution(**solution 3**), shake immediately for 10min, and then centrifuge: 10min / 3000g / 15℃.

----Take 4ml of the supernatant liquid to a 50ml polystyrene centrifuge tube, add 2ml of 2M NaCl (**solution** 1) and 7ml of ethyl acetate, shake completely for 10min, centrifuge: 5min / 3000g / 15℃.

----Transfer all the supernate into a 10ml clean glass tube, dry with nitrogen gas stream with 50-60^{o}C water bath.

----Add 1ml of 0.02M PBS (**solution** 2), vortex for 1min, and then add 1ml of n-hexane, vortex for 2min. Then transfer to a 2ml centrifuge tube, centrifuge: 5min / 3000g / 15℃.

----Remove the supernatant n-hexane phase, and take 50μl of the substrate water phase for assay.

Note: *if the sample concentration is out of the standard curve range, please dilute it with 0.02M PBS in the volume ratio of 1:5.*

Dilution factor: ***1***

Low detection limit

8.2.2 Method B (pork, liver)

----Homogenize the sample with homogenizer.

----Weigh 2.0±0.05g of homogenate to a 50ml polystyrene centrifuge tube, add 10ml of extraction solution (**solution** 4), shake thoroughly for 10min. And then incubate for 30min at 37℃. After that, centrifuge for 5min /3000g / 10℃.

----Take 50μl of the supernatant layer for assay.

Dilution factor: ***5***

8.2.4 Method C (chicken, liver)

----Homogenize the sample with homogenizer.

----Weigh 2.0±0.05g of homogenate to a 50ml polystyrene centrifuge tube, add 10ml of extraction solution (**solution** 4), add 5ml of n-hexane, shake thoroughly for 10min. After that, centrifuge for 10min/ 3000g /10℃.

----Remove the supernatant n-hexane phase, and take 100μl of the substrate layer into a 1.5ml tube, add 100μl of 0.02M PBS (**solution** 2), mix completely.

----Take 50μl of the prepared solution for assay.

Dilution factor: ***10***

8.3 Serum

----Keep the blood sample at room temperature for more than 30min till the serum separates. Then centrifuge:10min / 3000g / 10^{o}C.

----Take 1ml of the supernatant serum to a 10ml dry and clean glass tube, add 3ml of 0.02M BPS (**solution** 2), and mix completely.

----Take 50μl of the prepared solution for assay.

Note: *serum can be stored at 4℃ for 2 days, and stored for 2 weeks in freeze (-20^{o}C.)*

Dilution factor: ***4***

8.4 Honey

----Weigh 1.0±0.05g of sample to a 50ml polystyrene centrifuge tube, add 2ml of 0.02M PBS (**solution** 2), vortex to dissolve.

----Add 8ml of ethyl acetate, shake immediately for 10min. After that, centrifuge: 10min / 3000g / ambient temperature.

----Take 4ml of the supernatant liquid to a 10ml clean test tube, dry it with 50-60℃ water bath under nitrogen flow.

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----Add 1ml of 0.02M PBS (**solution** 2), vortex for 30s to dissolve.

----Take 50μl of the prepared solution for assay.

Dilution factor: 2

8.5 Urine

----Centrifuge urine sample: 3000g / ambient temperature / 5min, till the sample is transparent.

----Then take 1ml of the supernate into a 10ml dry and clean glass tube, add 3ml of extraction solution (**solution** 4), mix completely.

----Take 50μl of the prepared solution for assay.

Dilution factor: 4

8.6 Milk

----Centrifuge milk sample: 10℃ / 10min / 3000g, then remove the supernatant fat layer.

----Take 1ml of defatted milk sample, dilute with 19ml of 0.02M PBS (**solution** 2), and mix completely.

----Take 50μl of the prepared solution for assay.

Dilution factor: 20

9. Assay process

9.1 Notice before assay:

9.1.1 Make sure all reagents and micro wells are all at room temperature (20-25℃).

9.1.2 Return all the rest reagents to 2-8℃ immediately after used.

9.1.3 Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the repetitiveness of the ELISA analysis.

9.1.4 Avoid the light and cover the microwells during incubation.

9.2 Assay Steps:

9.2.1 Take all reagents out at room temperature (20-25℃) for more than 30min, shake gently before use.

9.2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8℃ immediately.

9.2.3 The diluted wash solution should be rewarmed to be at room temperature before use.

9.2.4 **Number:** Numbered every microwell positions and all standards and samples should be run in duplicate. Record the standards and samples positions.

9.2.5 **Add standard /sample, enzyme conjugate/ antibody**: Add 50μl of standard solution(**kit provided**) or prepared sample to corresponding wells. Add 50μl of enzyme conjugate(**kit provided**), 50μl of antibody solution(**kit provided**). Mix gently by rocking the plate manually and incubate for 60min at 25℃ with cover.

9.2.6 **Wash**: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250μl diluted wash solution (**solution** 5) at interval of 10s for 4-5 times. Absorb the residual water with absorbent paper (*the rest air bubble can be eliminated with unused tip*).

9.2.7 **Coloration**: Add 50μl of solution A(**kit provided**) and 50μl of solution B(**kit provided**) to each well. Mix gently by rocking the plate manually and incubate for 30min at 25℃ with cover (see 12.8).

9.2.8 **Measure**: Add 50μl of the stop solution(**kit provided**) to each well. Mix gently by rocking the plate manually and measure the absorbance at 450nm (It's suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution.)

10. Results

10.1 Percentage absorbance

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbance (\%)} = \frac{B}{B_0} \times 100\%$$

B ——absorbance standard (or sample)

B_0 ——absorbance zero standard

10.2 Standard Curve

To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the SDM standards solution (ppb) as x-axis.

The SDM concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution rate of each sample followed, and the actual concentration of sample is obtained.

Please notice:

Special software has been developed for result calculation, which can be provided on request.

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11. Sensitivity, accuracy and precision

Sensitivity: 1 ppb

Detection limit

Animal tissues (pork, chicken, liver), egg, honey…..…..1ppb

Serum / urine……..……4ppb

Milk……..……20ppb

Accuracy

Animal tissues (pork, chicken, liver) / urine…..…….. 75±10%

Egg…….69±10%

Honey, milk, serum……..……70±10%

Precision

Variation coefficient of the ELISA kit is less than 10%.

12. Notice

12.1 The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25℃).

12.2 Do not allow microwells to dry between steps to avoid unsuccessful repetitiveness and operate the next step immediately after tap the microwells holder.

12.3. Shake each reagent gently before using.

12.4. Keep your skin away from the stop solution for it is 0.5M H_2SO4 solution.

12.5 Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.

12.6 Keep the ELISA kits at 2-8℃, do not freeze. Seal rest microwell plates Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.

12.7 Substrate solution should be abandoned if it turns colors. The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5(A450nm<0.5).

12.8 The coloration reaction need 30min after the addition of solution A and solution B, and you can prolong the incubation time ranges from 35min to more if the color is too light to be determined. Never exceed 40min, on the contrary, shorten the incubation time properly.

12.9 The optimal reaction temperature is 25℃. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.

13. Storage

Storage condition: 2-8℃.

Storage period: 12 months

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