Sulfonamides ELISA Kit

Cat.#: DEIA021 Size: 96T

1. Background

Sulfonamides (SAs) are broadly applied bacteriophages, 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 45min 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. SA 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 SAs residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, SAs residue quantity in the sample can be calculated.

3. Applications

This kit can be used in quantitative and qualitative analysis of SAs residue (SMZ, SDM, SMM, SQX, SD/SDZ, SM_2 , etc.) in animal tissue.

4. Cross-reactions

Sulfamethoxazole (SMZ) ……100%
Sulfadiazine(SD/SDZ)……139%
Sulfamethazine(SM_2)……324%
Sulfadimethoxypyrimidine(SDM)……165%
Sulphadoxine(SDM2)……125%
Sulfamethizole(SMT)……82%
Sulfaquinoxaline(SQX)……257%
Sulfamerazine(SM_1)……283%
Sulfamethoxypyridazine(SMP)……187%
Sulfachloropyridazine (SPDZ)……400%
Sulfamonomethoxine (SMM)…… …318%
Sulfabenzamine(SB/SML)……117%
Sulfamethoxydiazine(SMD) ……505%
Sulfafurazole(SIZ)……513%
Sulfamethoxypyridazine(SMPZ)……235%
Sulfachlorpyrazine……282%
Phthalysulfathiazole(PST)……248%
Sulfathiazole(ST)……41%
Sulfacetamide(SA)……28%
Sulfapyridine(SPD)……6%
Sulfanitran……4%

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, 200ml, 500ml
- 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)
- Disodium phosphate dodecahydrate ($Na_2HPO_4.12H_2O$, AR)
- Sodium phosphate dibasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$, AR)
- Deionized water

6. Kit Components

- Microtiter plate with 96 wells coated with antigen
- Standard solutions(6 bottles×1ml/bottle)

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0ppb, 1ppb, 3ppb, 9ppb, 27ppb, 81ppb

- Spiking standard solution: (1ml/bottle) **1ppm**
- Concentrated enzyme conjugate 1 ml……..…red cap
- Enzyme conjugate diluent 10ml……....….green cap
- Substrate solution A 7ml……..……..white cap
- Substrate Solution B 7ml ……....red cap
- Stop solution 7ml….....…….....yellow cap
- 20×Concentrated wash solution 40ml .....….…..…transparent cap
- 2×Concentrated extraction solution 50ml…..blue cap

7. Reagents Preparation

Solution 1: Acetonitrile-ethyl acetate

Mix 50ml of acetonitrile and 50ml of ethyl acetate completely.

Solution 2: 0.2M PBS

Dissolve 25.8g of $Na_2HPO_4.12H_2O$ and 4.35g of $NaH_2PO_4 \cdot 2H_2O$ with 500ml of deionized water, mix completely.

Solution 3: 0.18M PBS (pH=6.98)

Dissolve 23.22g of $Na_2HPO_4.12H_2O$ and 3.91g of $NaH_2PO_4 \cdot 2H_2O$ with 500ml of deionized water, mix completely.

Solution 4: Extraction solution

Dilute the 2×concentrated extraction solution with deionized water in the volume ration of 1:1, 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, 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 that are treated with other methods can be stored at 4℃ for 24 hours.

8.2 Animal tissue(High detection limit method)

-----Homogenize the sample, take 2.0±0.05g homogenate to a 50ml polystyrene centrifuge tube, add 1ml of 0.2M PBS(**Solution 2**), vortex to paste, then add 7ml of acetonitrile-ethyl acetate(**Solution 1**), shake completely with shaker, then centrifuge for separation: 5min / 3000g / ambient temperature.

-----Take 4ml of the upper layer organic phase to a 10ml clean glass tube, dry with 50-60℃ water bath under nitrogen flow.

-----Add 1ml of n-hexane, vortex for 30s to dissolve the dry leftover, and then add 1ml of extraction solution(**solution 4**), vortex for 30s, transfer the solution into a 2ml polystyrene centrifuge tube, then centrifuge for separation: 5min / 3000g / ambient temperature.

-----Remove the upper layer organic phase, take 50μl of the lower layer aqueous phase for assay.

Dilution factor: 1

8.3 Animal tissue(Low detection limit method)

-----Homogenize the sample with homogenizer;

------Weigh 1.0±0.05g homogenate to a 50ml polystyrene centrifuge tube, add 10ml of 0.18M PBS(**Solution 3**), shake for 5min with shaker, then centrifuge for separation: 5min / 3000g / ambient temperature.

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

Dilution factor: 10

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

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standards and samples should be run in duplicate. Record the standards and samples positions.

9.2.5 **Add standard/sample**: Add 50µl of standard solution(**Kit component**) or prepared sample to corresponding wells.

9.2.6 Dilute the concentrated enzyme conjugate: Diluted the concentrated enzyme conjugate(**Kit component**) with the enzyme conjugate diluent(**Kit component**) in the volume ratio of 1:10(*e.g. 0.5ml of concentrated enzyme conjugate + 5ml of enzyme conjugate diluent*), mix completely.

9.2.7 **Add the diluted enzyme conjugate**: Add 50µl of the diluted enzyme conjugate per well, mix gently by rocking the plate manually and incubate for 30min at 25℃ with cover.

9.2.8 **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*).

9.2.8 **Coloration**: Add 50µl of solution A(**Kit component**) and 50µl of solution B(**Kit component**) to each well. Mix gently by rocking the plate manually and incubate for 15min at 25℃ with cover (see 12.8).

9.2.9 **Measure**: Add 50µl of the stop solution(**Kit component**) 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 SAs standards solution (ppb) as x-axis.

----The SAs 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 all data analysis, which can be provided on request.

11. Sensitivity, accuracy and precision

Sensitivity: 1ppb

Detection limit:

Animal Tissue(**High detection limit method**)……1ppb

Animal Tissue(**Low detection limit method**)…...…10ppb

Accuracy

Animal Tissue(**High detection limit method**)……100±20%

Animal Tissue(**Low detection limit method**)…... 100±20%

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 use.

12.4. Keep your skin away from the stop solution for it is the2M H_2SO_4.

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 15min after the addition of solution A and solution B; But you can prolong the incubation time ranges from 20-25min to more if the color is too light to be determined, never exceed 25min, on the

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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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