Version 10-09/22

User's Manual

Sulfadiazine ELISA Kit

REF **DEIANJ09**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Sulfadiazine(SDZ) Kit is for the quantitative detection of Sulfadiazine (SDZ) concentration in tissue, serum, honey, milk.

General Description

Sulfadiazine (SDZ), one kind of broad-spectrum antibiotic, is effective for animal production for its excellent antibacterial and pharmacokinetic properties. It has been widely used in different lines of poultry, cattle, agriculture and beekeeping. However, Sulfadiazine is prohibited in most countries as its residue may cause serious side effects, such as hematotoxic. Therefore, it is possible that sulfadiazine residues, after use in illegal practice, may lead to a risk for consumers.

Principles of Testing

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

Reagents And Materials Provided

Assay plate (96 wells) 1

Standards (40.5 ppb, 13.5 ppb, 4.5 ppb, 1.5 ppb, 0.5 ppb, 0 ppb) 6×1mL

Redissolving Solution (concentrate 2×) 2×20mL

Antibody 1×10mL

HRP-Conjugate 1×7mL

Wash Buffer (concentrate 20×) 1×20mL

Stop Solution 1×7mL

Substrate A 1×7mL

Substrate B 1×7mL

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Materials Required But Not Supplied

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at the

dual-wavelength 450/630 nm

- Equipments: microplate reader, printer, homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipets, balance (a reciprocal sensibility of 0.01 g)

- Micropipettors: single-channel 20-200 μL, 100-1000 μL, and multi-channel 250 μL

- Reagents: NaOH, ethyl acetate, n-Hexane, HCl (approx 36.5%), Na2HPO4·12H2O, NaH2PO4·2H2O, Acetonitrile (CH3CN)

Storage

Store at 2 - 8°C.

【SAMPLE STORAGE】

1. Untreated samples are stored at frozen environment.
2. Prepared sample can be stable at 2-8°C for 1 week.

Specimen Collection And Preparation

Instructions (The following points must be dealt with before the pre-treatment of any kind of sample):

1. This product can detect the following tissue samples:animal tissue, poultry, and aquatic tissue, eg: chicken, duck, beef, rabbit, fish, shrimp, etc.
2. Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.
3. 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. The 2×concentrated redissolving solution is diluted with deionized water at 1:1 (1 mL concentrated redissolving solution + 1 mL deionized water), used for sample redissolving.
2. 0.02 M PB buffer: dissolve 2.58g Na2HPO4 · 12H2O and 0.44g NaH2PO4·2H2O in the deionized water to 500 mL.
3. 0.5 M HCl: dissolve 4.3 mL HCl (36%) in deionized water to 100 mL.
4. 0.2 M NaOH: dissolve 0.8 g NaOH in deionized water to 100 mL.
5. Washing buffer: dilute 20 mL of the concentrated washing buffer (20×concentrated) with the distilled or deionized water at 1:19 to 400 mL (or just to the required volume) for use.

Samples preparation:

Tissue;

1. Homogenize the sample at 10000 r/min for 1 minutes.
2. Weigh 3 ± 0.05 g of the homogenized sample, put into centrifugal tube, add 3 mL 0.02 M PB buffer, shake properly. Then add 4 mL Ethyl acetate and 2 mL Acetonitrile, shake properly for 5 minutes, centrifuge at above 4000 r/min at room temperature (20-25°C) for 10 minutes.
3. Transfer 2 mL of the upper layer organic phase into a new centrifugal tube, blow to dry with nitrogen or air completely by rotary evaporation at 56°C.

4. Add 1 mL N-hexane, then add 1 mL of the diluted redissolving solution, shake strongly for 30s. Centrifuge at 4000 r/min at room temperature (20-25°C) for 5 minutes, remove the upper layer.
5. Take 50 µL of lower for further analysis.

Fold of dilution of sample: 1

Serum

1. Place blood sample at room temperature (20-25°C) for 30 minutes, centrifuge at above 4000 r/min at 10°C for 10 minutes, separate or filter serum.
2. Take 1 mL serum, add 3 mL 0.02 M PB buffer, mix properly for 30s.
3. Take 50 µL for further analysis.

Fold of dilution of the sample: 4

Honey

1. Weight 1.0±0.05 g honey into 50 mL centrifugal tube, then add 1 mL 0.5 M HCl. Be static at 37°C for 30 minutes.
2. Add 2.5 mL 0.2 M NaOH (adjust pH to 5), then add 4 mL Ethyl acetate, shake for 5 minutes, centrifuge at above 4000 r/min at room temperature (20-25°C) for 10 minutes.
3. Transfer 2 mL of the upper layer organic phase into a new centrifugal tube, blow to dry with nitrogen or air completely by rotary evaporation at 56°C, add 0.5 mL of the diluted redissolving solution, redissolve it for 30s.
4. Take 50 µL for further analysis.

Fold of dilution of the sample: 1

Milk

1. Take 0.1 mL milk, add 0.02 M PB buffer, dilute at 1:19(V/V) (20 µL milk + 380 µL 0.02 M PB buffer), shake for 30s.
2. Take 50 µL for further analysis.

Fold of dilution of the sample: 20

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Bring test kit to the room temperature (20-25°C) for at least 30 minutes, note that each reagent must be shaken evenly before use; put the required micro-well strips into plate frames. Re-sealed the unused microplate, stored at 2-8°C, not frozen.
2. Add 20 µL of the sample or standard solution into separate wells; add 50 µL of HRP-Conjugate, then add 80 µL of the antibody working solution into each well, mix gently by shaking the plate manully. Seal the microplate with the adhesive films, and incubate at 37°C for 30 minutes.
3. Pour liquid out of microwell, add 250 µL/well of washing buffer to wash microplate for 15-30s, repeat 4-5 times, flap to dry with absorbent paper at last.
4. Add 50 µL of Substrate A and 50 µL of Substrate B to each well, mix well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark. Avoid placing the plate in

direct light.

5. Add 50 µL of Stop Solution to each well. when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
6. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 630 nm. Subtract readings at 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Calculation

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 SDZ concentration in the sample.

1) Qualitative determination

The concentration range (ppb) of SDZ can be obtained from comparing the average OD value of the sample with that of the standard solution.

2) Quantitative determination

The mean values of the absorbance values obtained for 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/B0 ×100%

B—the average (double wells) OD value of the sample or the standard solution

B0—the average OD value of the 0 ppb standard solution

Draw the standard curve with the absorption percentages of standard solutions and the semilogarithm values of SDZ standard solutions (ppb) 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 dilution fold, finally obtaining SDZ concentration in the sample.

Using the professional software of this kit will be more convenient for accurate and rapid analysis of a large amount of samples.

Precision

Intra-Assay: CV<10%

Inter-Assay: CV<12%

Detection Range

0.5 ppb - 40.5 ppb

Detection Limit

Tissue, Honey 0.5 ppb

Serum 2 ppb

Milk 10 ppb

Sensitivity

0.5 ppb

Specificity

Sulfamethoxazole (SMZ) 100%

Sulfathiazole (ST) 7.8%

Sulfamerazine (SM1) 13%

Sulphadimidine (SM2) 21%

Precautions

1. The instruction manual also suits for the kit of 48T, but all reagents of 48T kit are reduced by half.
2. There may be some foggy substance in the wells when the plate is opened at the first time. It will not effect on the final assay results. Do not remove microtiter plate from the storage bag unless needed.
3. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
4. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as far as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
5. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
6. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
7. Controlling of reaction time: Observe the change of color after adding Substrate Solution (e.g. observation once every 10 minutes), Substrate Solution should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

8. Substrate Solution is easily contaminated. Substrate Solution should remain colorless or light blue until added to the plate. Please protect it from light.
9. Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
10. Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism.
11. Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbance measurement.
12. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.
13. Limited by the current condition and scientific technology, we can't completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit.
14. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
15. Kits from different batches may be a little different in detection range, sensitivity and color developing time.
16. Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.
17. Kits from different manufacturers with the same item might produce different results, since we haven't compared our products with other manufacturers.
18. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
19. Valid period: six months.

Limitations

- FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

- The kit should not be used beyond the expiration date on the kit label.

- Do not mix or substitute reagents with those from other lots or sources.

- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.

- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.

- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the ELISA Kit, the possibility of interference

cannot be excluded.

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