Version 09-05/24

User's Manual

Sulfamethoxazole ELISA Test Kit

REF **DEIASL127**

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

Sulfamethoxazole ELISA Test Kit is a competitive enzyme immunoassay for the quantitative analysis of Sulfonamide in eggs, fish, shrimp, meat (beef, chicken and pork), honey, milk, milk powder.

The unique features of the kit are:

(1) Rapid extraction method for various samples with high recovery (80-120%).

(2) High reproducibility.

(3) A quick ELISA assay (less than 1 hours regardless of number of samples).

Principles of Testing

The method is based on a competitive colorimetric ELISA assay. The drug of interest has been coated in the plate wells. During the analysis, sample and the Sulfamethoxazole antibody are added along with secondary antibody, tagged with a peroxidase enzyme. If the drug residue is present in the sample, it will compete for the Sulfamethoxazole antibody, thereby preventing the antibody from binding to the drug attached to the well. The resulting color intensity, after addition of the HRP substrate (TMB), has an inverse relationship with the drug residue concentration in the sample.

Reagents And Materials Provided

Sulfamethoxazole ELISA Test Kit has the capacity for 96 determinations of 42 samples in duplicate (assuming 12 wells for standards). Return any unused microwells to the foil bag and reseal them with the desiccant provided in the original package.

1. Sulfamethoxazole Plate: 1x96-well
2. Sulfamethoxazole Standards: 0.5ng/mL, 1.5 ng/mL, 4.5 ng/mL, 13.5ng/mL, 40.5 ng/mL, 1000 ng/mL, 1.0 mL for each.
3. Negative control: 1.0 mL
4. Sulfamethoxazole Antibody #1: 6 mL
5. HRP Conjugate Antibody #2: 6 mL
6. 10X Sample Extraction Buffer: 30 mL
7. 20X Wash Solution: 30 mL
9. Stop Buffer: 12 mL
10. TMB Substrate: 12 mL

* If you are not planning to use the kit for over 1 month, store Sulfamethoxazole Antibody #1, HRP Conjugate Antibody #2 at -20°C or in a freezer.

Materials Required But Not Supplied

1. Tissue Mixer (e.g. Omni TissueMaster Homogenizer)

2. Electronic balance
3. 10, 20, 100 and 1000 µL pipettes
4. Multi-channel pipette: 50-300 µL (Optional)
5. Vortex
6. Centrifuge
7. Incubator
8. Microtiter plate reader (450 nm)
9. Ethyl Acetate
10. N-hexane

Storage

Store the kit at 2-8°C. The shelf life is 12 months when the kit is properly stored.

Specimen Collection And Preparation

Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days.

Freeze samples to a minimum of -20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temps (20 - 25°C / 68 - 77°F) or in a refrigerator before use.

1.Preparation of 1X Sample Extraction Buffer:

Mix 1 volume of 10X Sample Extraction Buffer with 9 volumes of distilled water.

Egg

1. Weigh out 3 g of homogenized egg sample and mix with 6 mL of ethyl acetate, vortex 5 minutes at max speed.
2. Centrifuge for 5 minutes at 4,000 x g at room temperature (20 - 25°C / 68 - 77°F).
3. Transfer 2 mL of the supernatant (corresponding to 1 g of the original sample) into a new vial and use a rotary evaporator to dry the sample in a 50-60°C water bath under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 50-60°C water bath.
4. Dissolve the dried residue in 2 mL of n-hexane.
5. Add 1 mL of 1X Sample Extraction Buffer and mix by vortexing at maximum speed for 2 minutes.
6. Centrifuge for 5 minutes at 4,000 x g at room temp. Discard the upper hexane layer.
7. Use 50 µL of the lower aqueous layer per well for the assay.

Note: Dilution factor: 1.

If needed, the sample obtained from step 5 can be further diluted with 1X Sample Dilution Buffer.

Fish/Shrimp/Meat/Liver/Kidney

1. Remove fat from the sample. Homogenize the sample with a suitable mixer.
2. Weigh out 3 g of homogenized sample, mix with 6 mL of ethyl acetate, vortex 5 minutes at max speed.

3. Centrifuge for 5 minutes at 4,000 x g at room temperature (20 - 25°C / 68 - 77°F).
4. Transfer 2 mL of supernatant into a new centrifugal tube, blow to dry with nitrogen or dry completely by rotary evaporation at 50-60°C.
5. Dissolve the dried residue in 2 mL of n-hexane.
6. Add 1 mL of 1X Sample Extraction Buffer, shake for 2 minutes, and centrifuge at 4000 rpm at room temperature for 5 minutes. Remove the upper layer.
7. Use 50 µL of lower layer sample per well for the assay.

Note: Dilution factor: 1.

If needed, the sample obtained from step 6 can be further diluted with 1X Sample Dilution Buffer.

Honey

1. Weigh out 1 g of honey sample, mix with 9 mL of 1X Sample Extraction Buffer and vortex 5 minutes at max speed.
2. Use 50 µL of the sample per well for the assay.

Note: Dilution factor: 10.

Milk

1. For fat-free milk, take 50 µL of sample per well for the assay.
2. For the regular milk with fat, centrifuge the milk sample at 4,000 x g for 5 minutes, discard the upper fat layer, take 50 µL of sample per well for the assay.

Note: Dilution factor: 1.

If needed, the sample can be further diluted with 1X Sample Dilution Buffer.

Milk Powder

1. For fat-free milk powderweigh out 1 g of the sample, add 5 mL of distilled water and dissolve by shaking.
2. Take 50 µL of sample per well for the assay.
3. For the regular milk powder with fat, weigh out 1 g of the sample, add 5 mL of distilled water and dissolve by shaking.
4. Centrifuge the sample at 4,000 x g for 5 minutes, discard the upper fat layer.
5. Take 50 µL of sample per well for the assay.

Note: Dilution factor: 5.

Reagent Preparation

IMPORTANT:

All reagents should be brought up to room temperature before use (1 - 2 hours at 20 - 25°C / 68 - 77°F); Make sure you read "Precautions". Solutions should be prepared just prior to ELISA test. All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Using disposable reservoirs when handling reagents can minimize the risk of contamination and is recommended.

Preparation of 1X Wash Solution

Mix 1 volume of the 20X Wash Solution with 19 volumes of distilled water.

Assay Procedure

Label the individual strips that will be used and aliquot reagents as the following example:

Component	Volume per Reaction	24 Reactions
Sulfamethoxazole Antibody #1	50 µL	1.2 mL
HRP Conjugate Antibody #2	50 µL	1.2 mL
1X Wash Solution	2.0 mL	48 mL
Stop Buffer	100 µL	2.4 mL
TMB Substrate	100 µL	2.4 mL

1. Add 50 µL of each Sulfamethoxazole Standards in duplicate into different wells (Add standards to plate only in the order from low concentration to high concentration). Add 50 µL of each sample in duplicate into different sample wells.
2. Add 50 µL of HRP Conjugate Antibody #2 to each well.
3. Add 50 µL of Sulfamethoxazole Antibody #1 to each well and mix well by gently rocking the plate manually for 1 minute.
4. Incubate the plate for 30minute at room temperature (20 - 25°C / 68 - 77°F).
5. Wash the plate 5 times with 300 µL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).
6. Add 100 µL of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute while incubating (Do not put any substrate back to the original container to avoid any potential contamination. Any substrate solution exhibiting coloration is indicative of deterioration and should be discarded. Covering the microtiter plate while incubating is recommended).
7. After incubating for 15 minutes at room temperature (20 - 25°C / 68 - 77°F), add 100 µL of Stop Buffer to stop the enzyme reaction.
8. Read the plate as soon as possible following the addition of Stop Buffer on a plate reader with 450 nm wavelength (Before reading, use a lint-free wipe on the bottom of the plate to ensure no moisture or fingerprints interfere with the readings).

Calculation

A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a logarithmic curve.

$$\text{Relative absorbance (\%)} = \frac{\text{absorbance standard (or sample)x100}}{\text{absorbance zero standard}}$$

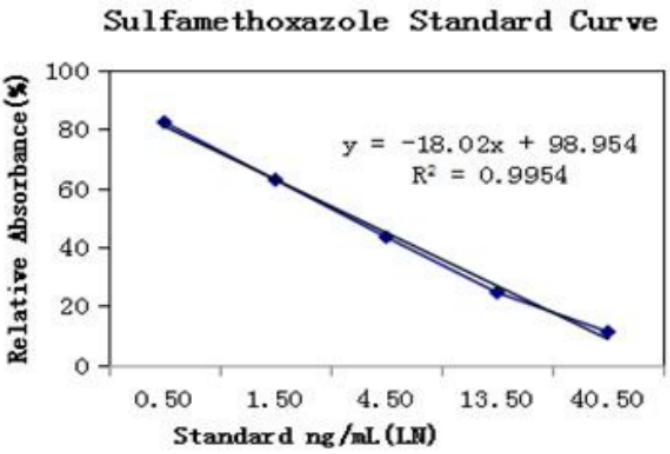
Use the mean relative absorbance values for each sample to determine the corresponding concentration of the tested drug in ng/mL from the standard curve.

Typical Standard Curve

The following figure is a typical Sulfamethoxazole standard curve. The sample detection and quantification limit are calculated as below.

Sample detection limit = (0.5 ng/g) x (dilution factor)

Sample quantification limit = (1.5ng/g) x (dilution factor)



Detection Limit

Egg: 0.5ppb

Fish/shrimp/meat/liver/kidney: 0.5ppb

Honey:5ppb

Milk:0.5ppb

Milk Powder:2.5ppb

Precautions

1. The standards contain Sulfamethoxazole. Handle with particular care.
2. Do not use the kit past the expiration date.
3. Do not intermix reagents from different kits or lots except for components with the same part No's within their expiration dates. ANTIBODIES, HRP CONJUGATE AND PLATES ARE KIT-AND LOT-SPECIFIC.
4. Try to maintain a laboratory temperature of 20°-25°C. Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
5. Make sure you are using only distilled or deionized water since water quality is very important.
6. When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.
7. Incubations of assay plates should be timed as precisely as possible. Be consistent when adding standards to the assay plate. Add your standards first and then your samples.
8. Add standards to plate only in the order from low concentration to high concentration as this will minimize the risk of compromising the standard curve. Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them equilibrate to room temperature (20 - 25°C / 68 - 77°F) while in the packaging.