



## User's Manual

# Sulfonamides ELISA Kit



DEIABL-QB13



96T



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.

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### 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](mailto:info@creative-diagnostics.com)  Web: [www.creative-diagnostics.com](http://www.creative-diagnostics.com)

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## PRODUCT INFORMATION

### Intended Use

This kit can be used in quantitative and qualitative analysis of SAs residue (SMZ, SDM, SMM, SQX, SD/SDZ, SM<sub>2</sub>, etc.) in honey.

### General Description

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 bacillus if they exist in human body for a long period. They also have potential carcinogenicity. 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.

### Principles of Testing

This kit is based on in-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.

### Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solutions(6 bottles×1ml/bottle)  
0ppb, 0.5ppb, 1.5ppb, 4.5ppb, 13.5ppb, 40.5ppb
3. Spiking standard solution: (1ml/bottle) 1ppm
4. Concentrated enzyme conjugate 1 ml.....red cap
5. Enzyme conjugate diluent 7ml.....green cap
6. Substrate solution A 7ml.....white cap
7. Substrate Solution B 7ml .....red cap
8. Stop solution 7ml.....yellow cap
9. 20×Concentrated wash solution 40ml.....transparent cap
10. 2×Concentrated extraction solution 50ml.....blue cap

### Materials Required But Not Supplied

#### 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: 500ml  
----Glass test tube: 10ml  
----Polystyrene Centrifuge tube: 2ml, 50ml  
----Micropipettes: 20ml-200ml, 100ml-1000ml, 250ml -multiple

### Reagents

----Acetonitrile (AR)  
----Deionized water  
----Concentrated hydrochloric acid (AR)

### Storage

Storage condition: 2-8°C.

Storage period: 12 months.

### Specimen Collection And Preparation

#### Notice and precautions before operation

1. Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.
2. Make sure that all experimental instruments are clean.
3. Samples that are treated with other methods can be stored at 4°C for 24 hours.

#### Honey

1. Take  $1.0 \pm 0.05$ g honey sample to a 50ml polystyrene centrifuge tube.
2. Add 1ml of 0.2M HCl solution (solution 1), shake completely.
3. Add 3ml of Acetonitrile, shake for 1min. then centrifuge for separation: 5min / 3000g / ambient temperature.
4. Take 1ml of the organic supernatant phase to 10ml clean and dry glass tube, dry in 50-60°C water bath with nitrogen gas stream or rotary evaporator.
5. Add 0.5ml of extraction solution (solution 2), vortex for 1min.
6. Take 50ml for assay.

Dilution factor: 2

## Reagent Preparation

### Solution 1: 0.2M HCl solution

Take 8.3 ml of Concentrated hydrochloric acid with deionized water to 500ml, mix completely.

### Solution 2: Extraction solution

Dilute the 2x concentrated extraction solution with deionized water in the volume ratio of 1:1, which will be used for sample extraction. This diluted solution can be conserved for 1 month at 4°C.

### Solution 3: Wash solution

Dilute the 20x concentrated wash solution with deionized water in the volume ratio of 1:19, which will be used to wash the plates. This diluted solution can be conserved for 1 month at 4°C.

## Assay Procedure

### Notice before assay

1. Make sure all reagents and micro wells are all at room temperature (20-25°C).
2. Return all the rest reagents to 2-8°C immediately after used.
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.
4. Avoid the light and cover the microwells during incubation.

### Assay Steps

1. Take all reagents out at room temperature (20-25°C) for more than 30min, shake gently before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. The concentrated extraction and wash solution should be rewarmed to be at room temperature before use.
4. Number: Numbered every microwell positions and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. Add standard/sample: Add 50µl of standard solution(Kit component) or prepared sample to corresponding wells.
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.
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°C with cover.

**Notice:** the mixed solution can't be stored, should be used immediately.

8. Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl diluted wash solution (solution 3) 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. 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°C with cover.

10. 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)

## Calculation

### 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<sub>0</sub> —absorbance zero standard

## Typical Standard Curve

1. 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.
2. 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.

## Precision

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

## Detection Limit

Honey: 2ppb

### Accuracy

Honey: 100±20%

## Sensitivity

0.5ppb

## Specificity

Sulfamethoxazole (SMZ) .....100%

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Sulfadiazine(SD/SDZ).....	208%
Sulfamethazine(SM <sub>2</sub> ).....	141%
Sulfadimethoxypyrimidine(SDM).....	600%
Sulphadoxine(SDM2).....	409%
Sulfamethizole(SMT).....	170%
Sulfaquinoxaline(SQX).....	277%
Sulfamerazine(SM1).....	227%
Sulfamethoxypyridazine(SMP).....	290%
Sulfachloropyridazine (SPDZ).....	200%
Sulfamonomethoxine (SMM).....	514%
Sulfabenzamine(SB/SML).....	10%
Sulfamethoxydiazine(SMD) .....	632%
Sulfafurazole(SIZ).....	368%
Sulfamethoxypyridazine(SMPZ).....	115%
Sulfachlorpyrazine.....	130%
Phthalysulfathiazole(PST).....	10%
Sulfathiazole(ST).....	41%
Sulfacetamide(SA).....	5%
Sulfapyridine(SPD).....	15%
Sulfanitran.....	4%

## Precautions

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°C).
2. Do not allow microwells to dry between steps to avoid unsuccessful repetitiveness and operate the next step immediately after tap the microwells holder.
3. Shake each reagent gently before use.
4. Keep your skin away from the stop solution for it is the 2M H<sub>2</sub>SO<sub>4</sub>.
5. Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.
6. Keep the ELISA kits at 2-8°C, do not freeze. Seal rest microwell plates. Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.
7. Substrate solution should be abandoned if it turns colors. The reagents may be turned bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A<sub>450nm</sub><0.5).
8. The coloration reaction needs 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 contrary, shorten the incubation time properly.

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