

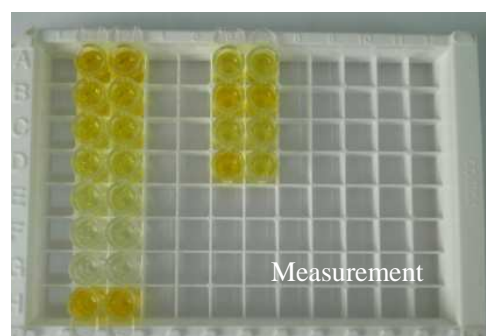
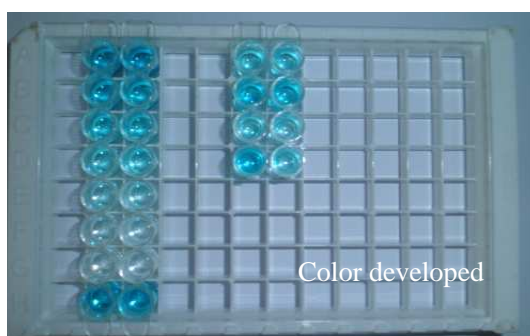
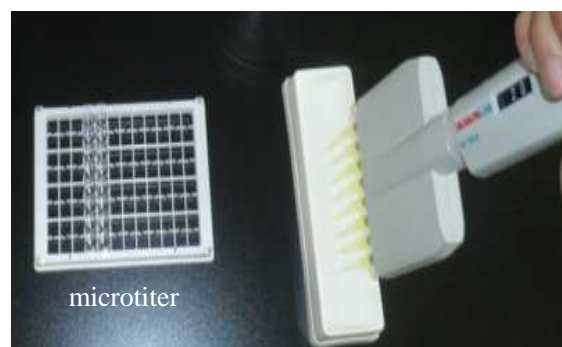
## ELISA Kit for Detection of Sulfamethazine

(Product Number: 5501E206)

(Sulfamethazine may be carcinogenic)

### INSTRUCTION MANUAL

(v. 1.00)



## 1. Introduction

Sulfonamides are widely used as feed additives, mainly for fattening of calves and pigs. Combined with inhibitors of dihydrofolate reductase such as trimethoprim, tetroxoprim, or pyrimethamine sulfonamides are also used in veterinary medicine for the treatment of intestinal infections, mastitis, pulmonitis and other (systemic) diseases. Sulfonamide residues may therefore occur in food of animal origin such as meat and milk. Particularly the transmission of the carcinogenic sulfamethazine represents a threat to human health. According to the EU-Law a maximum residue limit for all substances of the sulfonamide-group of 100 ppb in muscle, fat, liver and kidney and of 100 ppb in milk is valid.

This manual establishes procedures for determining sulfamethazine in meats and milks. The test is a competitive direct ELISA that provides exact concentrations in parts per billion (ppb). Free sulfamethazine in the samples and controls competes with enzyme-labeled sulfamethazine (conjugate) for the antibody binding sites. After a wash step, substrate reacts with the bound enzyme conjugate to produce blue color. The addition of the stopping solution leads to a color change from blue to yellow. The measurement is performed photometrically at 450 nm.

### **Assay Sensitivity, Precision, Accuracy**

- 1) Sensitivity: 0.5ppb
- 2) Precision: <20%
- 3) Accuracy: 70-120% recovery rate

## 2. Kit Contents

- 1) Microtiter plate ( 8 wells×12) precoated with antibodies to mouse IgG.
- 2) 6×sulfamethazine standard solutions (1ml/each):0ng/ml, 0.5ng/ml, 2.0ng/ml, 5.0ng/ml, 20.0ng/ml, 50.0ng/ml  
Controls provided: 0ppb, 0.5ppb, 2.0ppb, 5.0ppb, 20.0ppb, 50.0ppb
- 3) Antibody cross-reactivity(100×): sulfamethazine (0.2ml)
- 4) Enzyme Conjugate(100×) (0.2ml)
- 5) Washing buffer(10×) (50ml)
- 6) Antibody dilute solution (15ml)

- 7) Diluent buffer A (40ml)
- 8) Diluent buffer B (40ml)
- 9) Diluent buffer C (40ml)
- 10) TMB (18ml)
- 11) Stopping solution (7ml)
- 12) Instruction Manual

**Materials and reagents required but not provided:**

- 1) 50, 100 and 200 $\mu$ l precision micropipette.
- 2) 50-200 $\mu$ l multichannel micropipette.
- 3) Microtitre plate reader with 450nm filter.
- 4) n-hexane
- 5) Acetonitrile
- 6) Ethyl acetate
- 7) 100ml graduated cylinder.
- 8) 50ml container.
- 9) Centrifuge (4000r/min) and vortex mixer
- 10) Shaker
- 11) Thermostat
- 12) Timer

### 3. Preparation of Working Solutions

- **sulfamethazine standard solutions:** ready to use.
- **100 $\times$  Enzyme Conjugate:** dilute 80 $\times$  with Diluent buffer A(1+79).
- **100 $\times$ Antibody cross-reactivity:** dilute 80 $\times$  with Diluent buffer A(1+79).
- **washing buffer:** dilute 10 $\times$  with distilled water (1+9).
- **TMB:** ready to use.
- **stopping solution:** ready to use.
- **80 percent acetonitrile /water:** Using a graduated cylinder, measure 800 ml of acetonitrile and place it into a clean carboy with spigot. Add 200 ml deionized or distilled water to the acetonitrile and shake

vigorously until it is completely mixed. Store this solution at room temperature in a tightly closed container until use.

## 4. Preparation of Samples

### Meat

- a. Remove fat from meat and homogenize the fat free meat with a mixer to a fine paste
- b. mix 5 g of homogenized sample vigorously with 20 ml of an acetonitrile/water mixture (80/20) for 20 min
- c. centrifuge: 10 min / 3000 g / 15°C
- d. dilute 3 ml of the supernatant with 3 ml of distilled water
- e. add 4.5 ml of ethyl acetate and mix 20 min for extraction
- f. centrifuge: 10 min / 3000 g / 15°C
- g. transfer the superior ethyl acetate layer into another centrifugal vial and evaporate completely to dryness
- h. dissolve the dried residue in 1.5 ml of ready to use diluent buffer C
- i. for further degreasing add 1.5 ml of n-hexane (n-heptane) and mix for 1 min
- j. centrifuge: 10 min / 3000 g / 15°C
- k. remove the upper hexane layer completely
- l. transfer the aqueous (lower) phase into a new vial and Evaporate to dryness under a nitrogen stream at approximately 50°C for 2-3 min.
- m. Dilute 100µl of the filtrate with 400 of Diluent buffer A
- n. use 50 µl of the aqueous phase per well in the test

### Milk

- a. centrifuge milk samples for degreasing: 10 min / 3000 g / 15°C
- b. Dilute 100µl of the filtrate with 900µl of Diluent buffer B.

## 5. Immunoassay Procedure

- a) Allow reagents, microwells, and sample extracts to reach room temperature prior to running the test.
- b) Insert a sufficient number of wells into the microwell holder for all standards and samples to

be tested.

- c) Using a new pipette tip for each standard and sample, pipette 50µl of standards and prepared sample to separate wells.
- d) Add 50µl of enzyme conjugate into each well.
- e) Add 50µl of anti-sulfamethazine antibody into each well.
- f) Incubate for 20 minutes at room temperature (37°C)
- g) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.
- h) Using a wash bottle, fill each well with washing buffer. Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 4 washes).
- i) Add 150µl of TMB to each well. Incubate for 10 minutes at room temperature (37°C). Cover the wells with a paper towel to protect them from light sources.
- j) Add 50µl of stop solution to each well.
- k) Read results using a microwell reader with a 450 nm Thermo Labsystems.

## 6. Calculation

Divide the mean absorbance value of standards and samples (B) by the mean absorbance value of the Maximum Binding (Bo) and multiply by 100. Maximum binding is thus made equal to 100% and the absorbance values are quoted in percentages:

$$\frac{\text{absorbance standard (or sample)}}{\text{absorbance maximum binding}} \times 100 = B / B_o \%$$

## 7. Results

Since the samples have been diluted prior to assay, the concentrations in ng/ml read from the standard curve must be multiplied by the respective dilution factor to obtain the effective sulfamethazine concentration in samples expressed in ppb (ng/ml or ng/g).

## 8. Cautions

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- 1) If ambient temperature is lower than 20°C or the temperature of the reagents and standard solutions is less than the ambient temperature (e.g., 20- 25°C ) when used, the testing results may have negative bias (lower than it should be).
- 2) If it takes too long time to dry the washed microtiter during the washing steps, the results may demonstrate nonlinear calibration plot and poor repeatability, thus once the microtiter is dried after the washing steps, the following step should be immediately implemented to prevent the undesirable occurrence.
- 3) The standard solutions and chromogenic solution contain light-sensitive substances, thus the solutions should not be exposed under direct light.
- 4) To gain excellent results, any solutions should be thoroughly mixed before added on microtiter and the microtiter should be completely cleaned in between each step.
- 5) The stopping solution is a very strong acidic solution, so users should avoid the solution directly contacting with skin.
- 6) Neither use expired reagents, nor dilutes reagents or employs reagents produced by varied companies; otherwise, only gain less sensitive results.
- 7) Do not interchange individual reagents between kits of different lot numbers.
- 8) The kit should be stored at 2-8°C but not be frozen. Place any unused microwells to their original foil bag and reseal them together with the desiccant provided.

## 9. Contact Information

### UC BIODEVICES CORP.

3652 Edison Way

Fremont, CA 94538

USA

Tel: 1-510-730-2598

Fax: 1-510-795-1795

[www.ucbiodevices.com](http://www.ucbiodevices.com)

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