

## 9. Precautions

- Do not use the kit after the stated expiry date.
- Do not interchange kit contents from different lots.
- Do not use the chips if the aluminum pouch is damaged.
- Add the solutions to the bottom of the wells in the microplate for sample mixing.
- Bring all reagents and specimens to room temperature (20-25°C) prior to use.
- Keep the pipette vertically insert into the bottom of the hole in the coverslip when dispensing. It is recommended to pipette additional volumes of the sample mixtures into the chambers if the chambers are not full.
- When washing chips ensure that the barcoded-side is facing opposite to the washing tube side with the label “CapitalBio”.
- Keep the chip clean and away from dust and other contaminants.
- Store the used chips at a cool, dry and dark place until scanning

## Information

**Chip Incubation.** For convenience and high signal reliability, slide incubation is best performed using a CapitalBio BioMixer™ II Microarray Hybridization Station (Cat. No. 120030) which helps to reduced edge-effects.

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# CapitalBio Veterinary Drug Residue Detection Array Kit

**Cat. No. 310010**

## User Manual

**CapitalBio Corporation**

## 8.5 Quantitative Limit

**Table 3 Quantitative Limits of the Assay**

Veterinary Drugs	Quantitative Limit ( $\mu\text{g}/\text{kg}$ )	
	In Tissue Samples	In Cow Milk
Chloramphenicol	0.3	0.3
Sulfadimidine	20	10
Sulfaquinoxaline	20	10
Sulfamethoxazole	20	10
Sulfisoxazole	20	10
Sulfamonomethoxine	20	20
Enrofloxacin	20	10
Streptomycin	150	100
Dihydrostreptomycin	150	100

## 3. Applicable sample types

CapitalBio Veterinary Drug Residue Detection Array Kit was developed for the quantitative analysis of veterinary residues from animal products. The kit as been comprehensively validated for several tissue types:

- Muscle and liver of pig and chicken
- Cow milk

## 4. Format

24 tests/kit

## 5. Contents of the Kit

The kit consists of 2 parts, Kit (A) and Kit (B).

### 5.1 Materials in Kit (A)

- Sample Preparation Solution A, 2 bottles containing 150 ml each
- Sample Preparation Solution B, 1 vial containing 10 ml
- Sample Preparation Solution C, 1 bottle containing 250 ml
- Washing Buffer  $10\times$  Concentrate, 1 bottle containing 130 ml
- Coverslips, 1 package containing 12 slips
- Sample Mixing Plate plus Glue Film, 1 package containing 1 set
- Product Manual, 1 copy

### 5.2 Materials in Kit (B)

- Protein Chips, 4 packages containing 1 slide each
- Standard Reference Solution, 6 vials containing 1 ml each
- Primary Antibody  $10\times$  Concentrate, 1 vial containing 75  $\mu\text{l}$
- Secondary Antibody  $10\times$  Concentrate, 1 vial containing 130  $\mu\text{l}$
- Antibody Dilution Buffer, 2 vials containing 1 ml each
- Blocking Buffer, 1 vial containing 1.5 ml
- Magnetic Bead Solution, 1 vial containing 1.7 ml

## 6. Storage and stability

Store Kit (A) at room temperature (20-25°C)

Store Kit (B) at 2-8°C. **DO NOT FREEZE.**

The kits will remain stable for at least 6 months from date of manufacture if handled properly.

## 8.3 Immunoassay

### 8.3.1 Preparation of working solutions

Table 2. Preparation of working solutions sufficient for 1 chip

Working Solutions	Washing Buffer	Primary Antibody	Secondary Antibody
Washing Buffer 10x Concentrate	20 ml	/	/
Distilled Water	180 ml	/	/
Primary Antibody 10x Concentrate	/	16 µl	/
Secondary Antibody 10x Concentrate	/	/	26 µl
Antibody Dilution Buffer	/	144 µl	234 µl

### 8.3.2 Preparation of reaction solutions


8.3.2.1 Separate the Sample Mixing Plate by scissors according to the number of chips used (12 wells/chip);

8.3.2.2 Preparation of **Standard Solutions** (6 for each chip): Combine 12 µl of **Primary Antibody Working Solution** and 12 µl of each of the 6 **Standard Reference Solutions** into the bottom of 6 plate wells;

8.3.2.3 Preparation of **Sample Solutions** (6 for each chip): Combine 12 µl of **Primary Antibody Working Solution** and 12 µl of each of the 6 **Sample Solutions** into the bottom of 6 plate wells;

8.3.2.4 Cut appropriate sized pieces of Glue Film and stick onto the top of the plate wells. Mix well on the Vortex.

### 8.3.3 Washing and Drying

8.3.3.1 Add **Washing Buffer** into the chip washing tubes till the solution surface reaches the sign ;

8.3.3.2 Remove the incubation cassette from the incubator. Discard the coverslips and slip the chip into the washing tube at right angles. Cap the tube and shake by hand for a few seconds. Take out the chip and dispose the waste washing solution;

Please read instructions carefully before use

Bring all reagents to room temperature before use (20-25°C).

The assay procedure steps must be performed at room temperature unless otherwise indicated.

## 8.1 Sample preparation

### 8.1.1 Tissue sample preparation

8.1.1.1 Cut approximately 50 g of fat free meat or liver and homogenize for 1 min until well minced;

8.1.1.2 Weigh 5.0 g of minced sample and add into a 50 ml centrifuge tube;

8.1.1.3 Add 10 ml of **Sample Preparation Solution A** and cap the tube tightly;

8.1.1.4 Vortex thoroughly for 2 min;

8.1.1.5 Immerse the tube into an 80°C heated water bath for 20 min;

8.1.1.6 Allow the tube to equilibrate to room temperature naturally or by immersing it into cool water. Centrifuge at 4,000 rpm or 2,000 g for 10 min;

8.1.1.7 Transfer 4.0 ml of solution from the middle of the supernatant into a 15 ml centrifuge tube. Add 140.0 µl (meat sample) or 120.0 µl (liver sample) of **Sample Preparation Solution B** and mix thoroughly;

8.1.1.8 Centrifuge at 4,000 rpm or 2,000g for 10 min;

*If chloramphenicol is to be detected, skip 8.1.1.9 and continue from 8.1.1.10 till the end; otherwise, sample preparation ends at 8.1.1.9.*

8.1.1.9 Transfer 100 µl of solution from the middle of the supernatant into a 0.5 ml centrifugal tube. Add 200 µl of **Sample Preparation Solution C** and mix thoroughly for further use;

8.1.1.10 Transfer 2.5 ml of solution from the middle of the supernatant into a 15 ml centrifuge tube. Add 7.5 ml of Sample Preparation Solution C and mix thoroughly;

8.1.1.11 Add 60 µl of **magnetic bead solution**. Shake gently for 1 h;

8.1.1.12 Place the tube onto a magnetic separation stand for a few minutes until magnetic particles in solution are captured to the tube wall. Remove the solution. Add 1 ml of **Sample Preparation Solution C** and mix well.

## 1. Introduction

CapitalBio Veterinary Drug Residue Detection Array Kit is developed for the quantitative analysis of veterinary residues from meat and poultry products. Nine different veterinary drug residue species can be detected simultaneously, including total sulfonamides (sulfadimidine, sulfaquinolaxine, sulfamethoxazole, sulfisoxazole, sulfamonomethoxine), total streptomycins (streptomycin and dihydrostreptomycin), enrofloxacin and chloramphenicol. The sulfadimidine and sulfaquinolaxine are both detected separately, whereas the analysis of sulfisoxazole and sulfamonomethoxine are determined by the cross-reactivity with the antibody for sulfamethoxazole, and the analysis of dihydrostreptomycin is determined by the cross-reactivity with the antibody of streptomycin.

**Table 1. List Result and Actual Detected Items**

List Result	Actual Detected Items
Total Sulfonamides	SIZ+SMM+SMZ+SQX+SM <sub>2</sub>
SMZ	SMZ+SIZ+SMM
SM <sub>2</sub>	SM <sub>2</sub>
ENR	ENR
CAP	CAP
STR	STR+DSTR

SMZ: Sulfamethoxazole

ENR: Enrofloxacin

STR: Streptomycin

SM<sub>2</sub>: Sulfadimidine

CAP: Chloramphenicol

DSTR: Dihydrostreptomycin

## 2. The Principle of the test

The kit is based on a antibody-antigen competitive fluorescence immunoassay. Protein conjugated veterinary drug molecules are first covalently immobilized on the chip surface. After pretreatment, the test sample solution is then applied on the chip surface. The veterinary drug molecules in the test sample solution and the protein conjugated drug molecules immobilized on the slide surface will compete for the target antibodies added in the test solution. After incubation, excess antibodies, antigens or non-binding complexes are washed away. After incubation with fluorescence-labeled antibodies and after another washing step to remove unbound label, the chip is scanned by a microarray scanner. Higher fluorescence intensity corresponds to a lower concentration of veterinary drug residues in the tissue sample.

## 8.6 Cross-reactivity

**Table 4 Cross-reactivity of antibodies**

	SM <sub>2</sub>	SQX	SMZ	ENR	STR	CAP
Sulfadimidine	100	--	--	--	--	--
Sulfamonomethoxine	--	--	50	--	--	--
Sulfamethoxazole	20	17	--	--	--	--
Sulfaquinolaxine	--	100	--	--	--	--
Sulfadiazine	--	--	21	--	--	--
Sulfathiazole	--	--	525	--	--	--
Sulfamethoxazole	--	--	100	--	--	--
Sulfaguanidine	--	--	--	--	--	--
Sulfisoxazole	--	--	90	--	--	--
Sulfamethoxypridazine	--	--	--	--	--	--
Sulfamerazine	8	--	4	--	--	--
Enrofloxacin	--	--	--	100	--	--
Ciprofloxacin	--	--	--	1	--	--
Norfloxacin	--	--	--	--	--	--
Chloramphenicol	--	--	--	--	--	100
Thiamphenicol	--	--	--	--	--	--
Dihydrostreptomycin	--	--	--	--	130	--
Streptomycin	--	--	--	--	100	--
Neomycin	--	--	--	--	--	--

SM<sub>2</sub>: Sulfadimidine

SMZ: Sulfamethoxazole

STR: Streptomycin

Column headtitle: antibody

"--": The cross-reactivity rate is less than 1%.

SQX: Sulfaquinolaxine

ENR: Enrofloxacin

CAP: Chloramphenicol

Row headtitle: antigen

## 7. Materials required but not supplied in this kit

- LuxScan™ 10K-B Microarray Scanner (CapitalBio, Cat. No. 100020)
- Software for Veterinary Residue Analysis
- Centrifuge (>3,000 rpm)
- Vortex mixer
- Micropipettes for 20 µl, 200 µl, 1000 µl and 5000 µl with compatible tips
- Incubation cassette (CapitalBio, Cat. No. 430020)
- Sample preparation tubes (50 ml)
- Shaker
- Magnetic separation stand fit for 1.5 ml and 15 ml tubes

## 8. Assay procedure

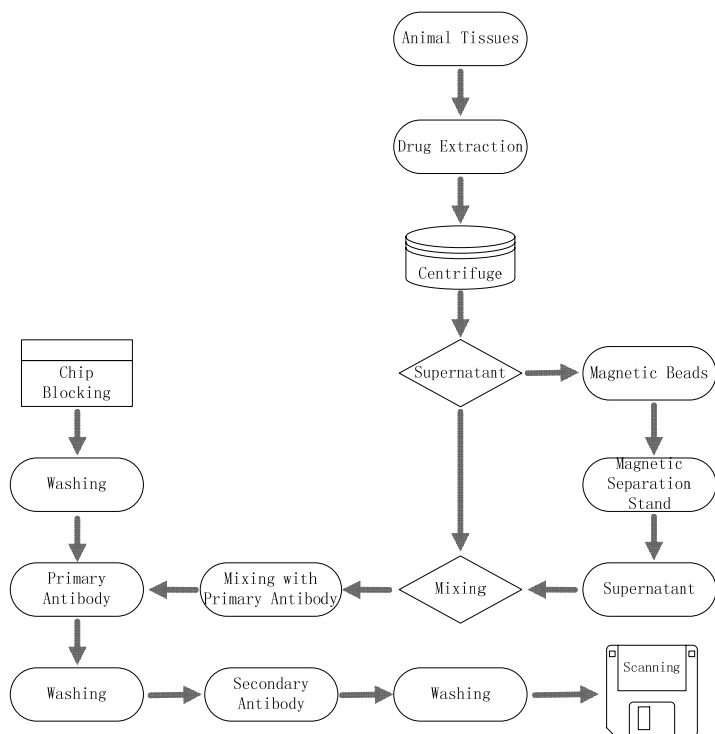


Figure 1. Veterinary Drug Residue Detection Flow Chart

8.3.3.3 Add **Washing Buffer** into the tubes again and slip the chip into the tube. Keep the barcoded-side facing opposite to the tube side with the label “CapitalBio”. Cap the tube tightly and shake on a shaker for 5 min with the tube side with the label “CapitalBio” facing up;

8.3.3.4 Remove the waste washing solutions and centrifuge at 1,000 rpm or 500 g for 2 min for chip drying.

### 8.3.4 Primary Antibody reaction

8.3.4.1 Place the chips into the incubation cassette. Keep the barcoded side facing up and place the coverslips on;

8.3.4.2 Pipette 20 µl of each of the **Standard Solutions** into the subarray No. 1-6 and 20 µl of each of the 6 **Sample Solutions** into the subarray No. 7-12. Cover the cassette and incubate at 37 °C for 30 min.

### 8.3.5 Washing and Drying (refer to 8.3.3)

### 8.3.6 Secondary Antibody reaction

8.3.6.1 Place the chips into the incubation cassette. Keep the barcoded-side facing up and place the coverslips on the chip;

8.3.6.2 Pipette 20 µl of diluted **Secondary Antibody Working Solution** into each well. Cover the cassette and incubate at 37 °C for 30 min.

### 8.3.7 Washing and Drying (refer to 8.3.3)

### 8.4 Chip Scanning (refer to the specification of the Veterinary Drug Residue Analysis Software)

Load the chip into the scanner holder and start scanning. The values for the concentrations of veterinary drugs in the samples are determined automatically by the software based on the calibration curve.

Separate the magnetic particles using the stand once again and dispose of the waste solution;

- 8.1.1.13 Add 1 ml of **Sample Preparation Solution C** and mix vigorously on a vortex. Transfer the entire solution into a 1.5 ml centrifugal tube. Separate the magnetic particles and dispose the waste solution with the stand;
- 8.1.1.14 Add 50  $\mu$ l of **Sample Preparation Solution C** and mix vigorously on a vortex until the magnetic particles in the tube are seen to be thoroughly suspended in solution;
- 8.1.1.15 Place the tube vertically in an 80°C heated water bath for 15 min;
- 8.1.1.16 Allow the tube to equilibrate to room temperature naturally or by immersing it into cool water. Mix vigorously on a vortex. Separate the magnetic particles by the stand or spin at 12,000 rpm in a microcentrifuge for 5 min to pellet the particles;
- 8.1.1.17 Combine 20  $\mu$ l of supernatant and 10  $\mu$ l of solution from the middle of supernatant (as in 8.1.1.8) into a 0.5ml tube and mix well.

### 8.1.2 Milk sample preparation

- 8.1.2.1 Prepare 200 ml of milk sample and mix well;
- 8.1.2.2 Weigh 5.0 g of mixed milk sample and add into a 50 ml centrifuge tube;
- 8.1.2.3 Add 5.0 ml of **Sample Preparation Solution A** and cap the tube tightly;
- 8.1.2.4 -8.1.2.6 Use the same procedures as described in 8.1.1.4-8.1.1.6;
- 8.1.2.7 Transfer 4.0 ml of solution from the middle of the supernatant into a 15 ml centrifuge tube. Add 80  $\mu$ l of **Sample Preparation Solution B** and mix thoroughly;
- 8.1.2.8-8.1.2.16 Use the same procedures as described in 8.1.1.8-8.1.1.16.

## 8.2 Chip Blocking

- 8.2.1 Place a protein chip incubation cassette on a level table and fill the reservoir wells in the base with distilled water to keep the chamber humid;
- 8.2.2 Determine the total number of chips needed and remove the appropriate number of chips from the package. Place the chips into the incubation cassette with the barcoded-side facing up and the barcode towards the operator. Place one coverslip on top of each of the chips with the sign “R→” down-right (**Figure 2**);
- 8.2.3 Pipette 20  $\mu$ l of blocking buffer through the injection hole vertically (**Figure 2**). Avoid forming bubbles. Cover the cassette and incubate at 37°C for at least 30 min. Reaction times longer than 3 h are not recommended.

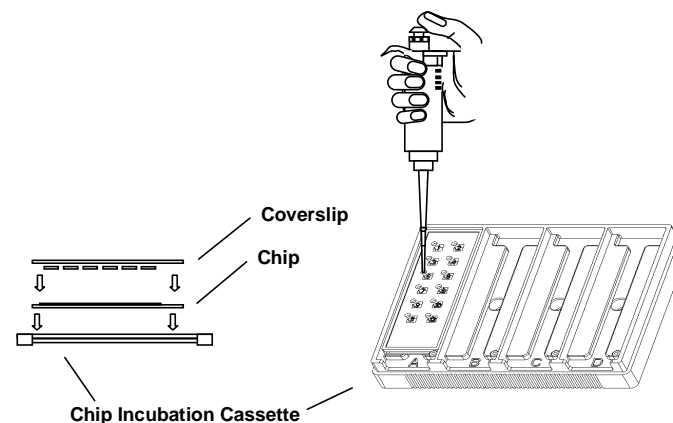


Figure 2. Schematic Diagram of Solution Addition