



## Technical Manual

### Dic (Diclazuril) ELISA Kit

- Catalogue Code: FSES0044
- Competitive ELISA Kit
- Research Use Only

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## Contents

1. Key features and Sample Types .....	3
2. Storage .....	3
3. Test Principle.....	3
4. Kit Contents.....	4
5. Experimental Preparation .....	5
6. Assay Procedure.....	6
7. Data Analysis .....	6
8. Notes .....	7

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## 1. Key features and Sample Types

### Sensitivity:

0.3 ppb (ng/mL)

### Assay Procedure:

25°C, 30 min ~15 min

### Detection Limit:

Tissue, Eggs - 15 ppb.

### Cross Reactivity:

Diclazuril - 100%, Toltrazuril, Ponazuril, Clazuril < 0.1%.

### Sample Recovery rate:

90%±30%.

### Storage:

2-8°C for 6 months.

### Expiry:

See Kit Label

## 2. Storage

Store at 2~8°C for 1 year. Avoid freeze.

Please store the opened kit at 2~8°C, the shelf life of the opened kit is up to 1 month.

## 3. Test Principle

This kit uses a Competitive-ELISA method. It can detect Diclazuril (Dic) in samples, such as tissue, eggs, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, Dic in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-Dic antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and substrate reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of Dic. The concentration of Dic in the samples can be calculated by comparing the OD of the samples to the standard curve.

## 4. Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	96-WellKit
1	ELISA Microtiter Plate	96 wells
2	Standards	1 mL each (0 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb, 24.3 ppb)
3	HRP Conjugate	7 mL
4	Antibody Working Solution	10 mL
5	Substrate Reagent A	6 mL
6	Substrate Reagent B	6 mL
7	Stop Solution	6 mL
8	20×Concentrated Wash Buffer	25 mL
9	20×Concentrated Sample Solution	25 mL
10	Plate Sealer	3 pieces
11	Sealed Bag	1 piece
12	Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

### Additional materials required:

#### Other materials required but not supplied

- **Instruments:** Microplate Reader, Homogenizer, Nitrogen Evaporators, Water bath, Vortex mixer, Centrifuge, Balance (sensitivity 0.01 g).
- **Micropipette:** Single-channel (20-200 µL, 100-1000 µL), Multi-channel (300 µL).
- **Reagents:** N-hexane, Acetonitrile, Sodium Chloride (NaCl), Acetic Acid (CH<sub>3</sub>COOH).

## 5. Experimental Preparation

Bring all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

### 1. Sample pre-treatment notice:

Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

### 2. Solution preparation

#### Solution 1: CH<sub>3</sub>COOH Solution

Take 1 mL **CH<sub>3</sub>COOH** and add 99 mL of deionized water, mix fully.

#### Solution 2: Wash Buffer

Dilute the **20xConcentrated Wash Buffer** with deionized water.  
(20xConcentrated Wash Buffer: Deionized water=1:19).

#### Solution 3: Sample Solution

Dilute the **20 × Concentrated Sample Solution** with deionized water. (20 × Concentrated Sample Solution: Deionized water=1:19)

### 3. Sample pre-treatment procedure

*Targets may be distributed unevenly, resulting in no detection. To avoid this, ensure to take sufficient samples when sampling.*

#### 3.1 Pre-treatment of tissue (livestock) sample:

1. Accurate weigh 1±0.05 g of homogenized sample into the 50 mL centrifuge tube, add 0.1 mL of **CH<sub>3</sub>COOH Solution** (Solution 1), 3 mL of **N-hexane** and 6 mL of **Acetonitrile**, then vortex for 1 min. Centrifuge at 4000 r/min for 5 min.
2. Discard the upper N-hexane layer liquid.
3. Take 0.5 mL supernatant to another centrifuge tube, dry at 50-60°C with nitrogen evaporators or water bath.
4. Dissolve the residue with 1 mL of **Sample Solution** (Solution 3) and mix fully for 30s.
5. Take 20 µL for analysis.

**Note: Sample dilution factor: 12, minimum detection limit: 15 ppb**

#### 3.2 Pre-treatment for egg sample:

1. Weigh 1±0.05 g of homogenate into 50 mL centrifuge tube, add 1 g **NaCl**, 1.5 mL of **CH<sub>3</sub>COOH Solution** (Solution 1), 3 mL of **N-hexane** and 4.5 mL of **Acetonitrile**, then vortex for 1 min. Centrifuge at 4000 r/min for 5 min.
2. Discard the upper N-hexane layer liquid.
3. Take 0.5 mL acetonitrile layer liquid to another centrifuge tube, dry at 50-60°C with nitrogen evaporators or water bath.
4. Dissolve the residue with 1 mL of **Sample Solution** (Solution 3), and mix fully for 30s.
5. Take 20 µL for analysis.

**Note: Sample dilution factor: 12, minimum detection limit: 15 ppb**

## 6. Assay Procedure

Bring all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2~8°C.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples must be tested in duplicate.**
2. **Add sample:** add 20 µL of **Standard or Sample** per well, add 50 µL of **HRP Conjugate**, then add 80 µL of **Antibody Working Solution** into each well. Cover the plate with plate sealer. Vortex for 10s gently to mix thoroughly. Incubate at 25°C for 30 min away from direct sunlight.
3. **Wash:** uncover the sealer carefully, remove the liquid of each well. Immediately add 260 µL of **Wash Buffer (Solution 2)** to each well and wash. Repeat wash procedure for 4 times, 30 sec intervals/time. Invert the plate and pat it against absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Colour Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50 µL of **Substrate Reagent B**. Gently vortex for 10s to mix thoroughly. Incubate at 25°C for 15 min away from direct sunlight. (The reaction time can be extended according to the actual colour change).
5. **Stop Reaction:** add 50 µL of **Stop Solution** to each well. Gently vortex for 10s to mix thoroughly.
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 5 min after stop reaction.

## 7. Data Analysis

1. **Absorbance (%) =  $A/A_0 \times 100\%$**

A: Average absorbance of standard solution or sample  $A_0$ :

Average absorbance of 0 ppb Standard solution

2. **Drawing and calculation of standard curve**

Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add the average absorbance value to standard curve to get corresponding concentration. **If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.**

For this kit, it is more convenient to use professional analysis form for accurate and fast analysis on a large number of samples.

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## 8. Notes

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25°C.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the FSES0044. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other FSES0044 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue colour. When OD value of standard (concentration: 0) < 0.8 unit (A450nm < 0.8), it indicates the reagents are deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

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