Version 26-04/24

User's Manual

Salinomycin ELISA Kit

REF **DEIA6841V2**

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.

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PRODUCT INFORMATION

Intended Use

Salinomycin ELISA Kit is a competitive enzyme immunoassay for the quantitative analysis of Salinomycin in meat, eggs and tissues.

Principles of Testing

This ELISA kit is based on the competitive binding enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with the analyte. During the reaction, the analyte in the sample or standard competes with antibody specific to the analyte. After the addition of enzyme conjugate, chromogenic substrate is used and the signal is measured by spectrophotometer. The concentration of the analyte in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Reagents And Materials Provided

1. Pre-coated Plate: 1 × 96-well plate (8 wells x 12 strips)
2. Standards: 5 vials (1mL/vial), 0ppb, 0.5ppb, 1.5ppb, 4.5ppb, 13.5ppb
3. Salinomycin Antibody: 1 vial (7mL)
4. Enzyme-Conjugated Antibody: 1 vial (12mL)
5. 10× Sample Dilution: 1 vial (10mL)
6. 20× Wash Solution: 1 vial (30mL)
7. Substrate solution A: 1 vial (7mL)
8. Substrate solutiob B: 1 vial (7mL)
9. Stop Solution: 1 vial (7mL)
10. Instruction
11. Protective foil
12. Self sealing bag

Materials Required But Not Supplied

1. Microplate reader (450nm detection wavelength filter, with optional 630nm correction wavelength filters).
2. Analytical balance (accuracy: 0.01 g)
3. Centrifuge (≥ 3000g)
4. Incubator (25°C)
5. Vortex mixer
6. Termovap sample concentrator
7. Homogenizer
8. Precision pipettes and disposable pipette tips

9. Timer
10. Deionized or distilled water
11. Ethyl Acetate (AR)

Storage

Store the kit and the kit reagents at 2-10°C, in a dry place and protected from the light.

Store unused strips in the Self sealing bag and keep the desiccant inside.

Specimen Collection And Preparation

Tissue, egg (Dilution factor: 5)

1. Weigh out 1.0±0.05 g of homogeneous sample into a 50ml centrifuge tube;
2. Add 5 ml of ethyl acetate and vortex for 30 s;
3. Centrifuge at room temperature above 3000 g for 5min;
4. Transfer 1ml upper organic phase to 2ml centrifuge tube, and dry under 60°C nitrogen flow;
5. Add 1 ml of 1× Sample Dilute and vortex samples for 30s;
6. Use 50µL of the sample for the assay.

Reagent Preparation

1× Sample Dilution: Samples are diluted with deionized water at the volume ratio of 1:9 (1 part of concentrated sample diluent +9 parts of deionized water).

1× Washing Solution: Washing solution is diluted with deionized water at the volume ratio of 1:19 (1 part of concentrated washing solution +19 parts of deionized water).

Assay Procedure

1. **Preparation:** Allow the microwell strips sealed inside the aluminium bag to reach room temperature. Withdraw an adequate number of strips and put the unused strips into the self sealing bag and placed at 4°C. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
2. **Add Sample:** Add 50 µl of the standards or diluted sample to the appropriate wells on the plate. Add 50µL of Salinomycin Antibody and mix well by gently rocking the plate manually for 30 seconds.
3. Incubate the plate for 30 minutes at room temperature (25°C)(Avoid direct sunlight and cold bench tops during the incubation. Covering the microtiter plate while incubating is recommended).
4. **Washing:** Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 250ul wash buffer and soak for 15-30s, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat 3-4 times.
5. Add 100 µL of enzyme conjugate solution. (Do NOT let the wells dry completely at any time.)
6. Incubate the plate for 30 minutes at room temperature (25°C)(Avoid direct sunlight and cold bench tops during the incubation. Covering the microtiter plate while incubating is recommended).

7. **Washing:** Empty the wells. Wash as described above.
8. Add 100 µL of the mixed solution of substrate A and substrate B into each well (**Note:** Substrate solution A and Substrate solution B should be mixed by volume 1:1, and must be fully mixed. The mixed solution shall be used within 10 minutes. Do not use metal to contain and stir reagents!).
9. Mix the solution by gently rocking the plate manually for 10s, cover the plate and incubate at 25°C in dark within 15-20 minutes.
10. Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
11. Measure the absorbance at 450 nm with a microplate reader within 5 minutes. It is recommended to use a reference reading at 630 nm.

Calculation

Percentage absorbance: The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

Absorbance (%) = $(B/B_0)*100\%$

B - absorbance of standards or samples

B_0 - absorbance of zero standard (0 ng/mL)

The semi-log system was used to substitute the percentage of absorbance corresponding to the standard substance into the standard curve, and the standard curve was fitted with the concentration of the standard substance.

Substituting the percentage of the absorbance of the sample to be detected into the fitted standard curve equation to obtain the concentration corresponding to the sample, and finally multiplying by the corresponding dilution multiple of the sample to obtain the content of the detected substance in the sample.

Precision

Intra-Assay: CV<5%

Inter-Assay: CV<15%

Detection Range

0.5ppb-13.5ppb (ng/ml or ng/g)

Detection Limit

Tissue/Egg: 10ppb (ng/ml or ng/g)

Sensitivity

0.5ppb (ng/ml or ng/g)

Recovery

Chicken/Duck: 100±30%

Egg: 80±30%

Precautions

1. Please read these instructions carefully before beginning this assay.
2. Bring all reagents and samples to room temperature (25±2°C) for 1h before use.
3. Reagent should be shaken before use and bubbles should be avoided during mixing.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Do not use the kit past the expiration date. Do not mix batches/lots from other orders of this kit, or from different kits.
6. Analyze the samples immediately after processing, otherwise the test results may be affected.
7. Substrate A liquid and substrate B liquid are colorless and transparent liquids. If they become blue before use or immediately after mixing, the reagents are contaminated or deteriorated.
8. On the premise of ensuring the accuracy, the sampling process must be rapid so as to avoid the influence of the reaction time difference on the test results.
9. Stop solution contains sulfuric acid, if accidentally spilled on the skin or clothing please immediately rinse with plenty of water. In case of eye contact, go to hospital for examination after thorough cleaning.

Limitations

This kit is quantitative or semi-quantitative and is recommended for screening and analysis of large numbers of samples. If the test result is positive, it is recommended to carry out confirmatory experiments (such as GC/MS, LC-MS/MS, etc.) using instrumental methods.