Cat.No: DEIA6841V2

Salinomycin ELISA Kit

1. Background

Salinomycin is commonly used as anti-coccidiosis in chicken. It leads to vasodilatation, especially coronary artery expansion and blood flow increment, which has no side effects on normal people, but for those who have got coronary artery diseases, it can be very dangerous.

HPLC is the common method of detecting salinomycin, and it costs too much compared with ELISA, which is more sensitive and precise and more appropriate for on-site supervision and mass screening.

This kit is a new product for drug residual detection based on ELISA technology, which is fast, easy to process, precise and sensitive, and it can considerably minimize operation errors and work intensity.

2. Test Principle

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Monensin residual in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme conjugate, tetramethyl-benzidine (TMB) substrate is used to show the color. Absorbance of the sample is negatively related to the salinomycin residue in it, after comparing with the Standard Curve, multiplied by the dilution factors, salinomycin residue quantity in the sample can be calculated.

3. Applications

This kit can be used in quantitative and qualitative analysis of salinomycin residue in animal tissues (muscle and liver) and eggs.

4. Cross-reactions

Salinomycin……..……..……..100%
Narasin……..……..……80%
Maduramicin ……..……..……<1%
Erythromycin ……..……..……<1%
Monesin…… ……..……..……<1%
Tylosin…….. ……..……..……<1%

5. Materials Required

5.1 Equipments

----Microtiter plate spectrophotometer (450nm/630nm)
----Rotary evaporator or nitrogen drying instruments
----Homogenizer
----Shaker
----Vortex mixer
----Centrifuge
----Analytical balance (inductance: 0.01g)
----Glass test tube: 10ml
----Polystyrene centrifuge tube: 2ml, 50ml
----Micropipettes: 20μl-200ul, 100μl-1000μl, 250μl-multipipette

5.2 Reagents

----Methanol (AR)
----Dichloromethane (AR)
----Sodium hydroxide (NaOH, AR)
----Sodium chloride (NaCl, AR)
----Acetonitrile (AR)
----Deionized water

6. Kit Components

- Microtiter plate with 96 wells coated with antigen
- Standard solutions(6 bottles:1ml/bottle)
  0ppb, 1ppb, 3ppb, 9ppb, 27ppb, 81ppb
- Spiking standard solution: (1ml/bottle) **1ppm**
- Enzyme conjugate 12ml……red cap
- Antibody solution 7ml…..……..……green cap
- Substrate solution A 7ml……..…..……white camp
- Substrate solution B 7ml……..……red cap
- Stop solution 7ml……..……yellow cap
- 20×concentrated wash solution 40ml ……transparent cap
- 2×Concentrated extraction solution 50ml…..…blue cap

7. Reagents Preparation

Solution 1: 8% NaCl solution

Dissolve 8.0g of NaCl with water and dilute to 100ml.

Solution 2: methanol- 8% NaCl solution

Mix 80ml of methanol with 20ml of 8% NaCl.

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Solution 3: 0.5M NaOH – 8%NaCl solution

Dissolve 2.0g of NaOH with 8%NaCl and dilute to 100ml with 8% NaCl solution;

Solution 4: Extraction solution

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

Solution 5: Wash solution

Dilute the concentrated wash solution with deionized water in the volume ratio of 1:19, which will be used to rinse the plates. The diluted wash solution can be conserved for a month at 4℃.

8. Sample Preparations

8.1 Notice and precautions before operation

(a) Please use one-off tips in the process of experiment, and change the tips when absorbing different reagent.

(b) Make sure that all experimental instruments are clean.

(c) Keep untreated samples in freeze;

(d) Treated samples should be tested immediately.

8.2 Animal tissue (muscle and liver)

----Homogenize the samples with homogenizer;

----Weigh 2.0±0.05g of the homogenate into a 50ml centrifuge tube, add 8ml of methanol-8% NaCl (**solution** 2), and shake for 5min;

----Centrifuge for separation: ambient temperature / 10min / 3000g;

----Transfer 2ml of the supernatant into a 50ml centrifuge tube, add 4ml of 0.5M NaOH-8% NaCl(**solution** 3), mix completely. Add 10ml of dichloromethane, shake for 5min;

----Centrifuge for separation: ambient temperature / 10min / 3000g;

----Remove the upper layer of organic phase, take 5ml of the down layer of aqueous phase into a 10ml clean glass test tube, dry with 50-60℃ water bath under nitrogen gas flow, then dissolve the dry leftover with 1ml of extraction solution(**solution** 4), vortex for 60s to dissolve completely;

----Take 50μl of the prepared solution for assay.

8.3 Eggs

----Mix the yolk and egg white completely;

----Take 1ml mixed egg sample into a 10ml centrifuge tube, add 5ml of acetonitrile, shake completely for 5min, and then centrifuge at room temperature (20-25℃) for 10min, at least 3000g;

----Transfer 1ml of the supernate into a 10ml clean glass test tube, dry with 50-60℃ water bath under nitrogen gas flow;

----Add 1ml of extraction solution (**solution** 4), vortex for 30s to dissolve completely;

----Take 200ul of the prepared solution and dilute with 600ul of extraction solution (**solution** 4), mix completely;

----Take 50μl of the prepared solution for assay.

9. Assay process

9.1 Notice before assay:

9.1.1 Make sure all reagents and microwells are all at room temperature (20-25 ℃).

9.1.2 Return all the rest reagents to 2-8 ℃ immediately after used.

9.1.3 Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the reproducibility of the ELISA analysis.

9.1.4 Avoid the light and cover the microwells during incubation.

9.2 Assay Steps

9.2.1 Take all reagents out at room temperature (20-25℃) for more than 30min, shake gently before use.

9.2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8℃ immediately.

9.2.3 The diluted wash solution should be rewarmed to be at room temperature before use.

9.2.4 **Number:** Number every microwell positions and all standards and samples should be run in duplicate. Record the standards and samples positions.

9.2.5 **Add standard solution/sample/antibody**: add 50μl of standard(**Kit component**) or prepared sample to corresponding wells, add 50μl of antibody solution(**Kit component**), mix gently by rocking the plate manually and incubate for 30min at 25℃ with cover.

9.2.6 **Wash**: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250μl of diluted wash solution (**solution** 5) 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*).

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9.2.7 **Add enzyme conjugate**: add 100ul of enzyme conjugate(**Kit component**) to each well, shake gently, and then incubate for 30min at 25℃ with cover. After that, take out the plate and repeat the ***wash step 9.2.6*** again.

9.2.8 **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℃ with cover(see 12.8).

9.2.9 **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*.)

10. Results

10.1 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.

$$\text{Absorbance}(\%) = \frac{B}{B_0} * 100\%$$

B ——absorbance standard (or sample)

B_0 ——absorbance zero standard

10.2 Standard Curve

----To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the monensin standards solution (ppb) as x-axis.

----The salinomycin concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

Please notice

Special software has been developed for all data reduction, which can be provided on request.

11. Sensitivity, accuracy and precision

Test Sensitivity: **1ppb**

Detection limit

Animal tissue……..……..5ppb

Eggs……..……..……20ppb

Accuracy

Tissue……..……..……100±30%

Eggs……...…..100±30%

Precision

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

12. Notice

12.1 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 ℃).

12.2 Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.

12.3. Shake each reagent gently before use.

12.4. Keep your skin away from the stop solution for it is 0.5M H_2SO_4 solution.

12.5 Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.

12.6 Keep the ELISA kits at 2-8 ℃,do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.

12.7 Substrate solution should be abandoned if it turns colors. The reagents may turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).

12.8 The coloration reaction need 15min after the addition of solution A and solution B; but you can prolong the incubation time if the color is too light to be determined. Never exceed 30min, on the contrary, shorten the incubation time properly.

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

13. Storage

Storage condition: 2-8℃.

Storage period: 12 months.

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