Version 20-04/24

User's Manual

Diclazuril ELISA Kit

REF **DEIABL-QB39**

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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This kit can be used in quantitative and qualitative analysis of Diclazuril residue in tissue (pork, chicken).

General Description

Diclazuril belongs to pyridines compounds. Because the Diclazuril has the broad-spectrum insect resistance, it becomes the most widely used coccidiostat. However, the irrational or intensive usage of diclazuril may result in residues in animal products, consequently not only cause potential hazards to human health but also increase drug resistance of coccidian.

This kit is a new product based on ELISA. It needs 45min in one operation. It can considerably minimize operation error and work intensity.

Principles of Testing

This ELISA kit is designed to detect Diclazuril based on the principle of "indirect-competitive" enzyme immunoassay. The microtiter wells are coated with coupling antigen. Colpidol in the sample competes with the coating antigen for binding to the limited number of antibody added. After the addition of a ready-to-use TMB substrate the signal is measured in an ELISA reader. The absorption is inversely proportional to the Diclazuril concentration in the sample.

Reagents And Materials Provided

1. Microtiter plate coated with antigen, 96wells
2. Standard solutions (5×1ml/bottle)
   0ppb,1ppb,3ppb,9ppb,27ppb
3. Spiking standard solution: (1ml/bottle) ……10ppm
4. Enzyme conjugate 7ml…….red cap
5. Antibody solution 7ml.....green cap
6. Solution A 7ml…….white cap
7. Solution B 7ml…….....red cap
8. Stop solution 7ml…….yellow cap
9. 20× Concentrated wash solution 40ml……transparent cap
10. 2× Concentrated extraction solution 50ml.....blue cap

Materials Required But Not Supplied

Equipments

1. Microtiter plate spectrophotometer (450nm/630nm)

2. Rotary evaporator / Nitrogen drying instrument
3. Homogenizer
4. Vortex mixer
5. Centrifuge
6. Analytical balance (inductance: 0.01g)
7. Graduated pipette: 10ml
8. Rubber pipette bulb
9. Volumetric flask: 500ml
10. Glass test tube: 10ml
11. Polystyrene Centrifuge tube: 50ml
12. Micropipettes: 20µl-200µl, 100µl-1000µl, 250µl- multipipette

Reagents

1. Acetonitrile (AR)
2. NaOH
3. Deionized water

Storage

Store at 2-8°C for 12 months.

Specimen Collection And Preparation

Notice and precautions before operation

1. Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.
2. Make sure that all experimental tools are clean.
3. The untreated sample should be stored in frozen.
4. The treated sample should be used immediately.
5. The samples showed turbid state after extraction, it doesn't influence the result.

Tissue (pork, chicken)----High LOD

1. Homogenize the sample.
2. Take 1.0±0.05g homogenized sample into a 50ml polystyrene centrifuge tube, add 1ml of 0.1M NaOH solution (solution 1) and vortex for 1min. Then add 7ml of acetonitrile and vortex for 2min. Centrifuge for separation: 3000g / 5min/ ambient temperature.
3. Take 1ml upper organic phase into 10ml clean dry glass tube. Dry in 50-60°C water bath under nitrogen flow (air).
4. Add 1ml extraction solution (solution 2) and vortex for 30s.
5. Take 50ul of the mix solution for assay.

Tissue (pork, chicken)----Low LOD

1. Homogenize the sample.

2. Take 1.0±0.05g homogenized sample into a 50ml polystyrene centrifuge tube, add 1ml of 0.1M NaOH solution (solution 1) and vortex for 1min. Then add 7ml of acetonitrile and vortex for 2min. Centrifuge for separation: 3000g / 5min/ ambient temperature.
3. Take 1ml upper organic phase into 10ml clean dry glass tube. Dry in 50-60°C water bath under nitrogen flow (air).
4. Add 1ml extraction solution (solution 2) and vortex for 30s.
5. Take 200 ul to 5ml clean dry glass tube ,add 600ul extraction solution (solution 2),vertex 10 S.
6. Take 50ul solution for assay.

Reagent Preparation

Solution 1: 0.1M NaOH solution

Weight 2g NaOH solution ,mix with 500ml deionized water completely .

Solution 2: Extraction solution:

Dilute the 2× concentrated extraction solution with deionized water in the volume ratio of 1:1(e.g. 10ml of 2× concentrated extraction solution + 10ml of deionized water), which will be used for dissolving the extracted sample. The diluted extraction solution can be conserved for 1 month at 4°C.

Solution3: Wash solution:

Dilute the 20× concentrated wash solution with deionized water in the volume ratio of 1:19(e.g. 5ml of 2× concentrated wash solution + 95ml of deionized water), which will be used for washing the microtiter plate. The wash solution can be conserved for 1 month at 4°C.

Assay Procedure

Notice before assay

1. Make sure all reagents and microwells are all at room temperature (20-25°C).
2. Return all the rest reagents to 2-8°C immediately after used.
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.
4. Avoid the light and cover the microwells during incubation.

Assay Steps

1. Take all reagents out at room temperature (20-25°C) for more than 30min, shake gently before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. The concentrated wash solution and concentrated extraction solution should be rewarmed before use.
4. **Number:** Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. **Add standard solution / sample:** Add 50µl of standard solution (kit insert) or prepared sample to corresponding wells. Then Add enzyme conjugate 50ul each wells ,add 50ul of antibody solution to each well. Shake gently. Incubate for 30min at 25°C with cover.
6. **Wash:** Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl of

wash solution (solution 3) at interval of 10s for 4-5 times. Absorb the residual water with absorbent paper (eliminate the air bubble with unused tips).

7. **Coloration:** Add 50µl of solution A (kit insert) and 50µl of solution B(kit insert) to each well. Mix gently by shaking the plate manually and incubate for 15 min at 25°C with cover.
8. **Measure:** Add 50µl of the stop solution (kit insert) to each well. Mix gently by shaking 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.).

Calculation

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.

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

B ——absorbance standard (or sample)

B_0 ——absorbance zero standard

Typical Standard Curve

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

The Diclazuril 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:

For data analysis of the ELISA kits, special software has been developed, which can be ordered on request.

Diluents ratio:

Tissue (High LOD)…….....…8

Tissue (Low LOD)…….……32

Precision

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

Accuracy:

Tissue.....……95±15%

Detection Limit

Tissue (High LOD) ……8ppb

Tissue (Low LOD)……..…32ppb

Sensitivity

Test Sensitivity: 1 ppb

Specificity

Diclazuril……100%

Monensin.....<0.1%

Maduramicin.....<0.1%

Salinomycin.....<0.1%

Precautions

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°C).
2. Do not allow microwells to dry between steps to avoid unsuccessful repetitiveness and operate the next step immediately after tap the microwells holder.
3. Shake each reagent gently before use.
4. The stop solution is high concentrated sulfuric acid. Don't touch it.
5. Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.
6. Keep the ELISA kits at 2-8°C, do not freeze. Seal rest microwell plates Avoid straight sunlight for the standard sample and the colorless chromogenic reagent are sensitive to light.
7. Substrate solution should be abandoned if it turns colors. The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5(OD450nm<0.5).
8. The coloration reaction needs 15min after adding Solution A and Solution B. And you can prolong the incubation time to 20min if the color is too light to be determined. Never exceed 25min, On the contrary, shorten the incubation time properly.
9. The optimal reaction temperature is 25°C. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.