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User's Manual

Dexamethasone ELISA Kit

REF **DEIABL-QB32**

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

This kit can be used in quantitative and qualitative analysis of dexamethasone residue in chicken and milk.

General Description

Dexamethasone is a glucocorticoid medicine. The hydrocortisone and prednisone is its ramification. It has the effect of anti-inflammatory, antitoxic, antiallergic, anti-rheumatism and the clinical application is wide.

The kit is a new generation of medicine residue test product based on the ELISA technology. It only needs 1.5 hours. It can reduce the operation error and the work strength.

Principles of Testing

This ELISA kit is designed to detect dexamethasone based on the principle of indirect-competitive enzyme immunoassay. The microtiter wells are coated with capture BSA-linked antigen. Dexamethasone in sample competes with the antigen coated on the microtiter plate for the antibody added. After the addition of enzyme conjugate, chromogenic substrate is used and the signal is measured by a spectrophotometer. The absorption is inversely proportional to dexamethasone concentration in the sample.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solutions (6×1mL/bottles): 0ppb, 0.1ppb,0.3ppb,0.9ppb,2.7ppb,8.1ppb
3. Spiking standard solution: 1mL/bottle, 100ppb
4. Enzyme conjugate: 12mL, red cap
5. Antibody solution: 7mL, green cap
6. Substrate solution A: 7mL, white cap
7. Substrate Solution B: 7mL, red cap
8. Stop solution: 7mL, yellow cap
9. 20×concentrated wash solution: 40ml, transparent cap
10. Extraction solution: 50ml, blue cap

Materials Required But Not Supplied

1. Equipments

Microtiter plate spectrophotometer (450nm/630nm)

Rotary evaporator or nitrogen drying instruments

Homogenizer

Shaker

Vortex mixer

Centrifuge

Analytical balance (inductance: 0.01g)

Graduated pipette: 10ml

Rubber pipette bulb

Volumetric flask: 500ml

Glass tube 10ml

Polystyrene centrifuge tube: 50ml

Micropipettes: 20ul-200ul, 200ul-1000ul

2. Reagents

N-hexane (AR)

Ethyl acetate (AR)

Deionized water

Storage

Storage condition: 2-8°C.

Storage period: 12 months.

Specimen Collection And Preparation

Notice and precautions before operation:

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

Make sure that all experimental instruments are clean.

Keep untreated samples in freeze;

When take the supernatant, don' t take the oily layer of fat.

Treated samples should be used immediately.

1. Chicken sample

a. Homogenize the samples with homogenizer;

b. Weigh 1.0±0.05g of the homogenate into a 50ml polystyrene centrifuge tube, add 8ml ethyl acetate and vortex for 5min.

c. Centrifuge: at 4000r/min / 5min/room temperature (20-25°C).

d. Take 2ml supernatant organic phase into 10ml clean dry glass tube. Dry with 50-60°C nitrogen gas or rotary evaporator;

e. Add 100ul n-hexane and vortex for 30s. Add 0.5ml extraction solution and vortex for 2min.

f. Centrifuge at room temperature (20-25°C) for 5min, at least 4000r/min;

g. Remove the supernatant organic phase, and take 50µl of the substrate phase for assay.

2. Milk

a. Take 1ml of milk sample into 50ml polystyrene centrifuge tube. Add 8ml ethyl acetate and vortex for 5min.

b. Centrifuge for separation: room temperature (20-25°C) /5min / 4000r/min;

c. Take 4ml supernatant organic phase into 10ml clean dry glass tube. Dry with 50-60°C nitrogen gas or rotary evaporator;

d. Add 100µl n-hexane and vortex for 30s. Add 0.5ml of extraction solution and vortex for 2min.

e. Centrifuge for separation: room temperature (20-25°C) /5min / 4000r/min;

f. Remove the supernatant organic phase, and take 50µl of the substrate phase for assay.

Reagent Preparation

Solution 1: Wash solution

Dilute the 20×concentrated wash solution with deionized water in the volume ration of 1:19, which will be used to wash the plates. This diluted solution can be conserved for 1 month at 4°C.

Assay Procedure

1. Notice before assay:

a. Make sure all reagents and microwells are all at room temperature (20-25°C).

b. Return all the rest reagents to 2-8°C immediately after used.

c. 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.

d. Avoid the light and cover the microwells during incubation.

2. Assay Steps

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

b. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.

c. The concentrated wash solution and extraction solution should be rewarmed to room temperature before use.

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

e. Add standard solution / sample and antibody solution: add 50µl of standard solution or prepared sample to corresponding wells. And add 50ul of antibody solution into each well. Shake gently. incubate for 30min at 25°C with cover (or in dark place)

f. Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl of diluted wash solution (solution 1) 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).

g. Add enzyme conjugate: add the enzyme conjugate 100µl into each well, mix gently by shaking the plate

manually and incubate for 30min at 25°C with cover. And repeat the step f.

h. Coloration: Add 50µl of substrate solution A and 50µl of substrate solution B into each well. Mix gently by rocking the plate manually and incubate for 15min at 25°C with cover(see Precautions).

i. Measure: Add 50µl the stop solution to each well. Mix gently by rocking the plate manually and measure the absorbance at 450nm (It is 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.

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

B ——absorbance standard (or sample)

B_0 ——absorbance zero standard

Standard Curve

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

The dexamethasone 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 analysis, which can be provided on request.

Dilute ratio:

Chicken: 2

Milk: 1

Performance Characteristics

Accuracy:

Chicken: 90±15%

Milk: 90±15%

Precision

CV of the ELISA kit is less than 10%.

Detection Limit

Chicken: 0.2ppb

Milk: 0.1ppb

Sensitivity

0.1ppb

Specificity

Dexamethasone: 100%

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 reproducibility and operate the next step immediately after tap the microwells holder.
3. Shake each reagent gently before use.
4. Keep your skin away from the stop solution for it is the H_2SO_4 solution.
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 kit at 2-8°C,do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.
7. Substrate solution should be abandoned if it turns colors. The reagents may be deteriorated if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).
8. The coloration reaction needs 15min after the addition of solution A and solution B; But you can prolong the incubation time to 20min or more 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.