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（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

DEX (Dexamethasone) ELISA Kit

Catalog No: E-FS-E009

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA)240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number(on the outside of the box) of the kit for more efficient service.

Test principle

This kit uses Indirect-Competitive-ELISA as the method. It can detect Dexamethasone(DEX) in samples, such as Muscle tissue, milketc. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with DEX. During the reaction, DEX in the samples or standard competes with DEX on the solid phase supporter for sites of DEX antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of DEX. You can calculate the concentration of DEX in the samples by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity:0.1ppb(ng/ml)

Reaction mode: 25℃，30min～30min～15min

Detection limit: Muscle tissue---0.2ppb, milk---0.5ppb，fodder---1ppb

Cross-reactivity: DEX ---100%

Sample recovery rate: Tissue/ milk/fodder---80%±15%,

Kits components

Item	Specifications
ELISA Micro-plate	96 wells
Standard Liquid	1mL each (0ppb,0.1ppb,0.3ppb,0.9ppb,2.7ppb,8.1ppb)
High ConcentratedStandard (100ppb)	1mL
HRP Conjugate(red cap)	11mL
Antibody Working Solution(blue cap)	5.5mL
Substrate Reagent A(white cap)	6mL
Substrate Reagent B(black cap)	6mL
Stop Solution(yellow cap)	6mL
20×Concentrated Wash Buffer(white cap)	40mL
2×RedissolvedBuffer (yellow cap)	50mL
Product Description	1 copy

Other supplies required

Instruments: Micro-plate reader, Printer, Homogenizer, Nitrogen Evaporators, Oscillators, Centrifuge, Graduated pipette, Balance(sensibility 0.01g).

High-precision transferpettor: single channel(20-200μL, 100-1000μL), Multichannel(300μL).

Reagents: Acetic ether, NaOH, N-hexane.

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate reader in advance, preheat the instrument, and set the testing parameters.

1. **Sample pretreatment Notice:**experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Solution preparation**

Solution 1: 2MNaOHsolution

Dissolve 40gNaOH to 500mL with deionized water

Solution 2: 0.3MNaOHsolution

Pipet75mL of 2MNaOHsolution to 500mL with deionized water

Solution 3: Redissolved Buffer

Double dilute the 2×redissolved bufferwith deionized waterfor the sample reconstitution, the redissolved buffercould be stored at 4℃ for one month.

Solution 4:1×Washing Buffer

Dilute 20×Concentrated Wash Buffer with deionized water

20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19

3. **Sample pretreatment procedure**

3.1 Pretreatment ofmuscle tissue:

(1) Add 2±0.05g sample to a 50mLcentrifuge tube, then add8ml acetic ether, oscillate for 5 minutes, centrifuge at 4000r/min at room temperature for 10min.

(2) Take 4mL supernatantto a new 50mLcentrifuge tube, then add 4ml 2MNaOH solution, oscillate for 5 minutes, centrifuge at 4000r/min at room temperature for 10min.

(3) Take 2mL upper liquid to another 10mLglass tube, blow-dry in nitrogen at 50-60℃.

(4) Dissolve the residual with1mLredissolved buffer, oscillate for 2 minutes.

(5) Take 100μL liquid foranalyze.

Note: Sample dilution factor: 5, minimum detection dose: 0.2ppb

3.2 Pretreatment ofmilk powder:

(1) Take 200μLmilk sample, add 0.8ml redissolved bufferand mix it.

(2) Take 100μL liquid foranalyze.

Note: Sample dilution factor: 5, minimum detection dose: 0.5ppb

3.3 Pretreatment of fodder:

(1) Take 1±0.05g milledfodder sample into a 50mLcentrifuge tube, then add 4mL 0.3M NAOH solution and oscillate, then add8ml acetic ether, oscillate for 5 minutes, centrifuge at 4000r/min at room temperature for 10min.

(2) Take 1mL of upper organic phaseliquid to another 10ml tube, blow-dry in nitrogen at 50-60℃.

(3) Dissolve the residual with 1mL n-hexane, add 1mL of reconstituted solution and oscillate for 2 min.

(4) Centrifuge at 4000r/min at room temperature for 5 min.

(5) Discard the upper liquid, take 100μLlower liquid foranalyze.

Note: Sample dilution factor: 8,minimum detection dose: 1ppb

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature before use.**All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**Dilute 40mL of concentrated wash buffer into 800mLwash working buffer with deionized or distilled water.

1. **Number:** number the samples and standard in order(multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50μL of Standard, Samples per well, then add 50μL of antibody working solution, cover the plate with sealer we provided, oscillate for 5s gently to mix thoroughly, incubate for 30 min at 25℃.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 250μL of washing buffer to each well and wash. Repeat wash procedure for 5 times, 30s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Add HRP conjugate:** Add 100 μL HRP conjugate to each well, incubate for 30 min at 25℃ in the dark.
5. **Wash:** Repeat step 3
6. **Color Development:** add 50μL of substrate solution A to each well, and then add 50μL of substrate solution B. Gently oscillate for 5s to mix thoroughly. Incubate shading light for 15min at 25℃(The reaction time can be extended according to the actual color change).
7. **Stop reaction:** add 50μL of stop solution to each well, oscillate gently to mix thoroughly.
8. **OD Measurement:** determine the optical density(OD value) of each wellat 450 nm with a micro-plate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10min after stop reaction.

Result analysis

1. **Absorbance (%)=A/A_0×100%**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0 ppb Standard

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 average absorbance value of sampleto 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 software for accurate and fast analysis on a large number of samples.

Notes

1. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperature is below25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor repeatability, move on to 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. Micro ELISA plate should be covered by plate sealer.Avoid the reagents to strong light.
5. Do not use expired kit and reagents of different batches of kits.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)is below0.5 unit(A_{450nm}＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, production date is on the packing box.