

QUALICHEK™ Dexamethasone ELISA

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RUO

Quantitative testing of Dexamethasone in samples such, as muscle tissue, milk, feed, etc.

RUO	For Research Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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Introduction:

Dexamethasone is a type of corticosteroid medication. It is used in the treatment of many conditions, including rheumatic problems, a number of skin diseases, severe allergies, asthma, chronic obstructive lung disease, croup, brain swelling, and along with antibiotics in tuberculosis.

Intended Use:

This kit is for quantitative testing for Dexamethasone in sample, such as muscle tissue, milk, feed, etc.

Principle:

This kit uses Indirect-Competitive-ELISA as the method. It can detect DEX in samples, such as muscle, tissue, milk, feed, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody, standard and other supplementary reagents. The microplate 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. The concentration of DEX in the samples can be calculated by comparing the OD of the samples to the standard curve.

Materials Provided:

1. Coated Microtiter plate - 1x 96 wells
2. Standards Liquid -1mL each (0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb)
3. High Concentrated Standard (100 ppb) - 1mL
4. HRP Conjugate - 11mL
5. Antibody Working Solution - 5.5 mL
6. Substrate A - 6 mL
7. Substrate B - 6 mL
8. Stop Solution - 6 mL
9. Wash Buffer (20X) - 40 mL
10. Plate Sealer - 3 pieces

Material required but not provided:

Instrument: Microtiter plate reader, Printer, Homogenizer, Nitrogen Evaporators/Water bath, Oscillators, centrifuge, Graduated pipette, Balance (sensitivity 0.01 g)

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

Reagents: Acetic ether, NaOH, N-hexane

Storage Information:

1. Store main kit components at 2-8°C Avoid freeze / thaw cycles.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Sample pretreatment:

Bring all reagents and samples to room temperature before use.

Open the microplate 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: 2 M NaOH solution; Dissolve 40 g NaOH to 500 mL with deionized water

Solution 2: 0.3 M NaOH solution; Pipet 75 mL of 2 M NaOH solution to 500 mL with deionized water

Solution 3: Reconstitution Buffer; Double dilute the 2xReconstitution buffer with deionized water for the sample reconstitution (2xReconstitution buffer(V) : deionized water(V)=1:1) , the reconstitution buffer could be stored at 4°C for one month.

Solution 4: Wash Buffer; Dilute 20xConcentrated Wash Buffer with deionized water. (20xConcentrated Wash Buffer (V): Deionized water (V) = 1:19)

3. Sample pretreatment procedure:

Pretreatment of muscle tissue:

1. Weigh 2 ± 0.05 g of homogenate sample to a 50 mL centrifuge tube, then add 8 mL of acetic ether, oscillate for 5 min, centrifuge at 4000 r/min for 10 min at room temperature.
2. Take 4 mL of supernatant to a new 50 mL centrifuge tube, then add 4 ml of 2 M NaOH solution, oscillate for 5 min, centrifuge at 4000 r/min for 10 min at room temperature.
3. Take 2 mL of upper liquid to another 10 mL glass tube, blow-dry in nitrogen evaporators/water bath at 50-60°C.
4. Dissolve the residual with 1 mL of Reconstitution buffer, oscillate for 2 min.
5. Take 100 μ L of liquid for analysis.

Note: Sample dilution factor: 2, minimum detection dose: 0.2 ppb

Pretreatment of milk powder:

1. Take 200 μ L of milk sample, add 0.8 mL of 1xReconstitution buffer and mix fully.
2. Take 100 μ L of liquid for analysis.

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

Pretreatment of feed:

1. Weigh 1 ± 0.05 g of milled feed sample into a 50 mL centrifuge tube, then add 4 mL of 0.3 M NaOH solution and oscillate, then add 8 mL of acetic ether, oscillate for 5 min, centrifuge at 4000 r/min at room temperature for 10 min.
2. Take 1 mL of upper organic phase liquid to another 10 mL glass tube, dry at 50-60 ° C with nitrogen evaporators/water bath.
3. Dissolve the residual with 1 mL of n-hexane, add 1 mL of Reconstitution buffer and oscillate for 2 min.
4. Centrifuge at 4000 r/min at room temperature for 5 min.
5. Discard the upper liquid, take 100 μ L of lower liquid for analysis.

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

Assay procedure:

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature for 30 min before use. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** add 100 µL of Standard or Sample per well, then add 50 µL of antibody working solution, cover the plate with sealer, oscillate for 5 s gently to mix thoroughly, incubate for 30 min at 25°C in the dark.
3. **Wash:** uncover the sealer carefully; remove the liquid in each well. Immediately add 300 µL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 s 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. **HRP Conjugate:** add 100µl of HRP conjugate to each well, incubate shading light for 30 min at 25°C
5. **Wash:** repeat step 3.
6. **Color Development:** add 50 µL of Substrate Reagent A to each well, and then add 50 µL of Substrate Reagent B. Gently oscillate for 5 s to mix thoroughly. Incubate shading light for 15 min at 25°C (The reaction time may be shortened or prolonged according to the depth of the color).
7. **Stop reaction:** add 50 µL of stop solution to each well, oscillate gently to mix thoroughly.
8. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Judgment of result:

$$\text{Absorbance (\%)} = A/A_0 \times 100\%$$

A: Average absorbance of standard or sample

A₀: Average absorbance of 0 ppb Standard

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 sample to 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. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25°C.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate 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. ELISA Microtiter plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit or reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard (concentration: 0) < 0.5 unit (A_{450nm} < 0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic; avoid contact with skin and eyes

Technical specification:**Sensitivity:** 0.1 ppb (ng/mL)**Reaction mode:** 25°C, 30 min~30 min~15 min**Detection limit:** Muscle tissue ---0.2 ppb,
milk---0.5 ppb, feed---1 ppb**Reactivity:** DEX---100%**Sample recovery rate:** Tissue/ milk / feed ---80%±15%**LIMITED WARRANTY**

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