Dexamethasone ELISA Kit

Catalog No.: abx364822

Size: 96T

Storage: Store at 4°C for up to 6 months. For long-term storage, the ELISA plate, Standards and Biotin conjugated antibody can be stored at -20°C.

Application: For quantitative detection of dexamethasone in muscle tissue, milk, serum, and feed.

Sensitivity: 0.1 ppb (ng/ml)

Detection Limit: Muscle Tissue – 0.2 ppb, Milk – 0.5 ppb, Feed – 1 ppb

Introduction: Dexamethasone is a synthetic corticosteroid, with a fluorine atom added to the ninth carbon. The chemical exhibits mild immunosuppressive functions, with uses ranging from preventing swelling during dental surgery, to treating anaphylaxis. It is also used to mitigate the adverse effects caused by some chemotherapy agents. Side effects of dexamethasone treatment include metabolic changes, cataracts, Cushing's syndrome, cardiomyopathy, and seizures.

Principle of the Assay

This kit is based on indirect-competitive enzyme-linked immuno-sorbent assay technology. Dexamethasone is pre-coated onto a 96-well plate. The standards and samples and a biotin conjugated antibody specific to dexamethasone are added to the wells and incubated. After washing away the unbound conjugates, avidin conjugated to Horseradish Peroxidase is added to each microplate well. After TMB substrate solution is added only wells that contain dexamethasone will produce a blue colour product that changes into yellow after adding the stop solution. The intensity of the yellow colour is inversely proportional to the dexamethasone amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of dexamethasone can be calculated.

Kit components

1. One pre-coated 96 well plate
2. Standards - 0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb: 1 ml each
3. HRP conjugate reagent: 11 ml
4. Antibody solution: 5.5 ml
5. Substrate reagent A: 6 ml
6. Substrate reagent B: 6 ml
7. Stop solution: 6 ml
8. Wash buffer (20X): 40 ml
9. Reconstitution buffer (2X): 50 ml
10. Plate sealer: 3 pieces
11. Hermetic bag: 1

Materials Required But Not Provided

1. 25°C incubator
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. 50 ml centrifuge tubes
7. Absorbent filter papers
8. 100 ml and 1 L graduated cylinders
9. Nitrogen evaporator or water bath
10. Homogenizer

Reagents Required But Not Provided

1. Sodium hydroxide
2. Ethyl acetate
3. Deionized water
4. N-hexane

Protocol

A. Preparation of sample and reagents

1. Preparation of Sample pretreatment solutions

- **Solution 1 – 2 M NaOH solution**
  Dissolve 40 g of NaOH in 500 ml deionized water. Mix thoroughly.

- **Solution 2 – 0.3 M NaOH solution**
  Dissolve 75 ml of solution 1 in 500 ml deionized water. Mix thoroughly.

- **Solution 3 – Working Reconstitution buffer solution (1X)**
  Dilute the 2X Reconstitution Buffer 2-fold with deionized water (i.e. dilute 50 ml 2X Reconstitution buffer in 50 ml deionized water) to make the 1X Working Reconstitution Buffer solution. The 1X solution can be stored at 4°C for up to one month.

- **Solution 4 – Working Wash buffer solution (1X)**
  Dilute the 20X Wash buffer 20-fold with deionized water (i.e. dilute 40 ml 20X Reconstitution buffer in 760 ml deionized water) to make the 1X Working Wash buffer solution.

2. Sample pretreatment

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Muscle Tissue:** Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Weigh 2 g of homogenised muscle tissue and add to a 50 ml centrifuge tube. Add 8 ml of ethyl acetate and vortex for 5 minutes. Centrifuge the homogenates at 4000 rpm for 10 min and collect 4 ml of supernatant. Transfer the supernatant to a new 50 ml tube, and add 4 ml of 2 M NaOH solution. Vortex for 5 minutes, and centrifuge the sample again at 4000 rpm for 10 min. Aspirate 2 ml of the upper fluid layer and add to a 10 ml glass tube. Dry in a nitrogen evaporator or water bath at 50 - 60°C. Dissolve the solid residue in 1 ml of Working Reconstitution buffer, and vortex for 2 minutes. Aliquot 100 µl and assay immediately.
  Note: Sample dilution factor: 2, minimum detection dose: 0.2 ppb.

- **Milk:** Add 800 µl of Working Reconstitution buffer solution to 200 µl of milk. Mix thoroughly, and aliquot 100 µl for analysis.
  Note: Sample dilution factor: 5, minimum detection dose: 0.5 ppb.

- **Feed:** Weigh 1 g of crushed feed sample and add to a 50 ml centrifuge tube. Add 4 ml of 0.3 M NaOH solution and vortex, then add 8 ml of ethyl acetate. Vortex for 5 minutes, then centrifuge at 4000 rpm for 10 minutes. Aspirate 1 ml of the upper fluid layer and add to a 10 ml glass tube. Dry in a nitrogen evaporator or water bath at 50 - 60°C. Dissolve the solid residue with 1 ml of n-hexane and 1 ml of reconstitution buffer and vortex for 2 minutes. Centrifuge at 4000 rpm for 5 minutes. Aspirate and discard the upper liquid phase. Aliquot 100 µl of the lower phase for analysis.
  Note: Sample dilution factor: 8, minimum detection dose: 1 ppb.

Note:

» Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.

» Please bring sample slowly to room temperature.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 100 µl of the standard solutions into the standard wells.
3. Add 100 µl of prepared sample into the sample wells.
4. Immediately add 50 µl of Antibody solution. Add the solution at the bottom of each well without touching the side wall.
5. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 25°C for 30 minutes in dark conditions.
6. Remove the cover, and wash the plate 5 times. Discard the solution without touching the side walls. Blot the plate on an absorbent material. Add 300 µl of Working Wash buffer to each well and soak for at least 1 min. Discard the contents and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure for a total of five times.

Note: For automated washing, discard the solution in all wells and wash five times overfilling the wells with Working Wash buffer. After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

7. Add 100 µl of HRP conjugate reagent into each well. Add the solution at the bottom of each well without touching the side wall.
8. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate in the dark at 25°C for 30 minutes.
9. Repeat the wash step as described in step 7.
10. Add 50 µl of substrate solution A into each well, and then 50 µl of substrate solution B into each well. Tap the plate gently to ensure thorough mixing. Cover the plate and incubate at 25°C in dark conditions for 15-20 minutes (incubation time is for reference only, do not exceed 30 minutes). When an apparent gradient appears in the standard wells the reaction can be terminated.
11. Add 50 µl of Stop solution into each well (including the blank well). There should be a colour change to yellow. Gently tap the plate to ensure thorough mixing.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

This assay is competitive, therefore there is an inverse correlation between dexamethasone concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration (y-axis) and absorbance measured (x-axis). Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1. Equilibrate all reagents to room temperature (18-25°C) prior to use and centrifuge the tubes briefly in case any contents are trapped in the lid.
2. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
3. The wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. Avoid foaming or bubbles when mixing or reconstituting components.
5. Do NOT let the plate dry out completely during the assay as this will inactivate the biological material on the plate.
6. Ensure plates are properly sealed or covered during incubation steps.

7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. To avoid cross contamination do not reuse pipette tips and tubes.
9. Do not use components from a different kit or expired ones.
10. Substrate solution A and substrate solution B are easily contaminated; work under sterile conditions when handling these solutions. The substrate solutions should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.