Corticosterone (Rat/Mouse) ELISA Kit

Cat. No.: DEIA2185
Pkg. Size: 96T

**Intended use**

The Corticosterone rat/mouse ELISA is a competitive immunoassay for measurement of corticosterone in rat and mouse serum or plasma.

**General Description**

Corticosterone (CORT) is a 21-carbon steroid hormone of the corticosteroid type produced in the cortex of the adrenal glands.

**Principle Of The Test**

The Corticosterone rat/mouse ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. An unknown amount of corticosterone present in the sample and a defined amount of corticosterone conjugated to horseradish peroxidase compete for the binding sites of corticosterone antiserum coated to the wells of a microplate. After incubation on a shaker the microplate is washed four times. After addition of the substrate solution the concentration of corticosterone is inversely proportional to the optical density measured.

**Reagents And Materials Provided**

1. Microtiterplate, 12 x 8 (break apart) strips with 96 wells; Wells coated with polyclonal rabbit anti-corticosterone antibody.
2. Calibrator 0, 1 vial, 0.3 mL, ready to use.
3. Calibrator (Calibrator 1–5), 5 vials, 0.3 mL each, ready to use; Concentrations: 15 – 50 – 185 – 640 – 2250 ng/mL.
4. Incubation Buffer, 1 vial 11 mL, ready to use.
5. Enzyme Conjugate, 1 vial, 7 mL, ready to use; Corticosterone conjugated to horseradish peroxidase.
6. Substrate Solution, 1 vial, 22 mL, ready to use; contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.
7. Stop Solution, 1 vial, 7 mL, ready to use; contains 2 N Hydrochloric Acid solution.
8. Wash Solution, 1 vial, 50 mL (10X concentrated).

**Materials Required But Not Supplied**

1. Centrifuge
2. A microtiter plate reader capable for endpoint measurement at 450 nm.
3. Microplate mixer operating more than 600 rpm.
4. Vortex mixer.
5. Calibrated variable precision micropipettes (10 μL, 50 μL, 100 μL, 200 μL).
6. Absorbent paper.
7. Distilled or deionized water.
8. Timer.
9. Semi logarithmic graph paper or software for data reduction.

**Storage**

Store all contents at 2 to 8 °C.
**Specimen Collection And Handling**

**Serum**
Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20 °C. Avoid repeated freeze-thaw cycles.

**Plasma**
Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Use in assay immediately or aliquot and store samples at -20 °C. Avoid repeated freeze-thaw cycles.

**Reagent Preparation**
All reagents should be at room temperature before use. Dilute 50 mL of 10X concentrated Wash Solution with 450 mL deionized water to a final volume of 500 mL. The diluted Wash Solution is stable for at least 3 months at room temperature.

**Assay Steps**
1. Prepare a sufficient number of microplate wells to accommodate calibrators and samples in duplicates.
2. Dispense 10 μL of each Calibrator and Sample with new disposable tips into appropriate wells.
3. Dispense 100 μL of Incubation Buffer into each well.
4. Add 50 μL Enzyme Conjugate into each well.
5. Incubate for 2 hours at room temperature on a microplate mixer.
   Important note Optimal reaction in this assay is markedly dependent on shaking of the microplate!
6. Discard the content of the wells and rinse the wells 4 times with diluted Wash Solution (300 μL per well). Remove as much Wash Solution as possible by beating the microplate on absorbent paper.
7. Add 200 μL of Substrate Solution to each well.
8. Incubate without shaking for 30 minutes in the dark.
9. Stop the reaction by adding 50 μL of Stop Solution to each well.
10. Determine the absorbance of each well at 450 nm. It is recommended to read the wells within 15 minutes.

**Calculation**
1. Calculate the average absorbance values for each set of calibrators, controls and samples.
2. Using semi logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration from the calibration curve.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log are recommended.
5. The concentration of the samples can be determined directly from this calibrator curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations, this dilution factor has to be taken into account.

**Typical Standard Curve**
Following data are intended for illustration only and should not be used to calculate results from another run.
### Detection Range

15 - 2250 ng/mL

### Sensitivity

4.5 ng/mL

### Precautions

1. TMB substrate has an irritant effect on skin and mucosa. In case of contact with skin or eyes, wash thoroughly with water. Please note that extreme temperature changes may cause spontaneous decay of the peroxide.

2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.

3. Do not mix reagents of different lots. Do not use expired reagents.

4. The microplate contains snap-off strips. Unused wells must be stored at 2 ℃ – 8 ℃ in the sealed foil pouch and used in the frame provided.

5. Avoid contact with Stop Solution. It may cause skin irritation and burns.

6. Pipetting of samples and reagents must be performed as quickly as possible and in the same sequence for each step.

7. Change pipette tips between samples, controls and reagents to avoid carry over contamination.

8. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.

9. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.

10. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

11. Assay reagents contain Proclin against microbial growth. In case of contact with eyes or skin, flush immediately with water.

12. All reagents should be at room temperature ( 21 ℃ – 26 ℃ ) before use. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.

### Limitations

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.
Analyte Gene Information

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<thead>
<tr>
<th><strong>Gene Name</strong></th>
<th>Cort cortistatin [Mus musculus]</th>
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<td><strong>Official Symbol</strong></td>
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<td><strong>Synonyms</strong></td>
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<tr>
<td><strong>Function</strong></td>
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REFERENCES