Cortisol (Cat) ELISA Kit

Catalog Number KA2307
96 assays
Version: 3.1

Intended for research use only
# Table of Contents

- **Introduction** .................................................................................................................. 3
  - Intended Use ...................................................................................................................... 3
  - Principle of the Assay ....................................................................................................... 3

- **General Information** ...................................................................................................... 4
  - Materials Supplied ........................................................................................................... 4
  - Storage Instruction .......................................................................................................... 4
  - Materials Required but Not Supplied ............................................................................... 4
  - Precautions for Use ......................................................................................................... 4

- **Assay Protocol** ............................................................................................................... 6
  - Reagent Preparation ........................................................................................................ 6
  - Sample Preparation ......................................................................................................... 6
  - Assay Procedure ............................................................................................................. 6

- **Data Analysis** ............................................................................................................... 7
  - Calculation of Results ..................................................................................................... 7
  - Performance Characteristics ............................................................................................ 7

- **Resources** ..................................................................................................................... 8
  - References ....................................................................................................................... 8
  - Plate Layout .................................................................................................................... 9
Introduction

Intended Use

The Cortisol (Cat) ELISA Kit is an immunoassay designed for the quantitative determination of cortisol in serum or plasma. The test is intended for the research of serum or plasma cortisol in feline and related species.

Principle of the Assay

The Cortisol (Cat) ELISA Kit is based on a widely used immunoassay technique. A sample (serum or plasma) containing an unknown amount of cortisol to be assayed (unlabeled antigen) is added to a standard amount of a labeled derivative of the same substance (labeled antigen). The labeled and unlabeled antigens are then allowed to compete for high affinity binding sites on a limited number of antibodies coated on to the plate. After washing away the free antigen, the amount of labeled antigen in the sample is reversibly proportional to the concentration of the unlabeled antigen. The actual concentrations in unknown samples are obtained by means of a standard curve based on known concentrations of unlabeled antigen analyzed in parallel with the unknowns. In this kit an enzyme label is used. The biospecific reaction takes place during 1 hour incubation. After washing away, substrate solution is added and the enzyme allowed to react for a fixed time before the reaction is terminated. Absorbencies are measured at 450 nm using ELISA plate reader. A standard curve is produced using values from 6 standards from which absorbency values for blank tubes have been subtracted. Results for unknown may be read directly from this standard curve using either manual calculation or by a suitable computer program.

This kit is suitable for the direct measurement of cortisol in serum or plasma samples. The assay is designed to measure circulating levels of cortisol in cats and related species and it is not advisable to use for other species.

Note: The cortisol levels should be established in your laboratory using your own set of samples and standards and good laboratory practice should be employed where applicable.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
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<tbody>
<tr>
<td>Microtiter wells coated with cortisol specific antibody</td>
<td>96 wells</td>
</tr>
<tr>
<td>Enzyme Conjugate</td>
<td>12 mL</td>
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<tr>
<td>Cortisol lyophilized Standards, (contains 0, 1, 2.5, 5, 10, 50, 200 ng/mL should be diluted using 0.6 mL Standard/Sample diluent).</td>
<td>7 vials</td>
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<tr>
<td>TMB Color Reagent</td>
<td>12 mL</td>
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<tr>
<td>Stop solution (2 N HCl)</td>
<td>6 mL</td>
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<tr>
<td>20x Wash Buffer</td>
<td>20 mL</td>
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<tr>
<td>Standard/Sample diluent</td>
<td>20 mL</td>
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</table>

Storage Instruction

✓ Store the kit at 4-8°C upon receipt and when it is not in use.
✓ Keep microtiter wells in a sealed bag with desiccants to minimize exposure to damp air.
✓ After every use place the caps tightly. Store reagents and reconstituted standards at 4-8°C.

Materials Required but Not Supplied

✓ Semiautomatic pipettes: 20 μL and 200 μL
✓ Disposable pipette tips
✓ Micortiter plate shaker
✓ Plate washer
✓ Absorbant paper
✓ 37°C incubator
✓ Parafilm to cover plate
✓ Distilled water
✓ A microtiter well reader with bandwidth of 10 nm or less and an optical density range of 0 to 2 OD or greater at 450 nm wavelength is acceptable for use in absorbency measurement.

Precautions for Use

This product is for research use only.
Please read the entire protocol carefully before starting your experiment.
This kit contains reagents manufactured from serum/plasma components. The source materials have been tasted by immunoassay for hepatitis B surface antigen and antibodies to HIV virus and found to be negative. Nevertheless, all blood products and samples should be considered potentially infectious and handling should be in accordance with the procedures defined by an appropriate biohazard safety guideline or regulations in your labs or local and state.

The contents of this kit, and their residues, must not come into contact ruminating animals or swine.

Avoid contact with the Stopping Reagent. It may cause skin irritation and burns.

Do not use reagents after expiration date.

Do not mix or use components from the kits with different lot numbers.

Replace caps on reagents immediately. Do not switch caps.

Reagents contain sodium azide (NaN₃) as a preservative. On disposal, flush with a large volume of water to prevent azide build-up.

Do not pipette reagents by mouth.

Do not use reagents from other kits or mix with other manufactured test kits.

Limitation of the test

The present ELISA is designed for helping the scientist to analyze test samples from cats only. There are no warranties, expressed, implied or otherwise indicated, which extend beyond this description of this product. Abnova is not liable for property or laboratory damage, personal injury, or test samples loss, or economic loss caused by this product. The analyst should establish the standard curve and a small number of samples before proceeding to analyze a large number of samples.
Assay Protocol

Reagent Preparation

✓ Allow all the kit contents to stand 30-60 minutes at room temperature before use.
✓ Read the instructions well and understand before starting the assay system.
✓ All the test procedures must be carried-out from start to finish without interruption.
✓ Use disposable tips for each sample and do not mix.
✓ Mix Wash buffer 1 part with 19 parts of distilled water.
✓ All lyophilized standards should be reconstituted in 0.6 mL using Standard/Sample diluent, these standards should be stored at 4-8°C or -20°C for long term use. Avoid thaw-freeze cycles.

Sample Preparation

✓ This kit is suitable for use with serum or plasma samples. The use of grossly hemolytic or lipemic samples should not be used may affect results. Samples with bilirubin may also interfere with the assay.
✓ A venous blood sample (enough to produce about 0.5 mL serum) is collected aseptically.
✓ Highly concentrated samples should be diluted with Standard/Sample diluent (eg.1:5, or 1:10), to bring on to a readable range on the curve.

Assay Procedure

1. Pipette 25 μL of standards.
2. Add 25 μL of test samples into appropriate wells.
3. Add 100 μL of Enzyme Conjugate Solution to each well (except those set aside for blanks).
4. Incubate for 1 hour at 37°C.
5. Terminate the reaction and wash the plate 4-5 times with Wash Solution (250-300 μL) per well. Invert plate, tap firmly against absorbent paper to remove any residual moisture.
6. Add 100 μL of TMB color reagent into each well (including the blanks). Remember for pipetting order.
7. Incubate the plate for 20 minutes without shaking.
8. Stop reaction by adding 50 μL of Stop Solution (a drop) to each well in the same sequence that the Substrate Solution was added. Gently mix for 1-2 minutes.
9. Read the absorbency at 450 nm with a microwell reader.

Note: The substrate incubation should be carried out at room temperature (within the temperature range 25-28°C). For temperature outside this range, the duration of the incubation should be adjusted by approximately 1 minute/1°C.
Data Analysis

Calculation of Results

✓ Calculate the mean absorbance values (A) for each set of reference standards, controls, samples and blanks.
✓ Subtract the value for blanks from those for standards, control and unknown samples.
✓ Calculate the B/BO% values by dividing each value by the value for the zero-standard.
✓ For the standards, plot a graph on semi-log graph paper with B/BO% values on the ordinate and the Cortisol concentrations (ng/mL) on the abscissa.
✓ Using the graph read off the Cortisol concentrations for the unknown samples.
✓ You may use any commercial assay software to analyze the data.

Performance Characteristics

• Sensitivity and Expected Values
  It is recommended that each laboratory should establish values to reflect differences specific to experimental conditions. The minimum detectable concentration of cortisol by this assay is estimated to be 1 ng/mL.
Resources

References

4. BONDY PK. The adrenal cortex, in Randy PT, Rosenberg LE., Metabolic control and disease (8 ed) 1980, WB Sanders, Philadelphia, p1427-1499.
Plate Layout

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