Cortisol (Bovine) ELISA Kit

Catalog Number KA2275

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

The Cortisol ELISA test is an immunoassay designed for the quantitative determination of Cortisol in serum/plasma or urine samples. The test is intended for professional use as an aid in the diagnosis and monitoring of physiological/pathological conditions related to serum/plasma Cortisol in bovine and related species.

Principle of the Assay

The Cortisol Quantitative Test is based on a widely used immunoassay technique. A sample (serum/ plasma/urine) 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 biospecfic 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/plasma/urine samples. All samples to be assayed must be diluted with sample dilution buffer (eg. 1part with 4 parts of sample buffer; 0.05 ml+0.2 ml). This system can also be applied for urine and salivary Cortisol detection. It may also be used following an extraction procedure for assaying tissue Cortisol (call for details of the procedure).

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

Component	Amount
Microtiter wells coated with Cortisol specific antibody	96 wells
HRP Enzyme Conjugate	12 ml
Cortisol Standards, Ready to use (Contains 0, 1, 2.5, 5, 10, 50, 200 ng/ml)	1 set, 0.5 ml/vial
TMB Color Reagent	12 ml
Stop Solution (2N HCl)	6 ml
20x Wash Buffer	20 ml
Sample Diluent	20 ml

Storage Instruction

Immediately after receiving the kit all standards, if not used, should be kept at -20℃. Unopened test kits should be stored at 2-8℃. The microtiter plate should always be kept in a sealed bag with desiccants to minimize exposure to damp air at room temperature. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. Do not leave any reagents at room temperature more than 3 hours.

Materials Required but Not Supplied

- ✓ Semiautomatic pipettes: 20 ul and 200 ul
- ✓ Disposable pipette tips
- ✓ Microtiter plate shaker
- ✓ Microtiter well reader
- ✓ Plate washer
- ✓ Absorbant paper
- ✓ 37℃ incubator
- ✓ Parafilm to cover plate
- ✓ Distilled water
- ✓ A microtiter plate reader with a bandwidth of 10nm or less, with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at a 450nm wavelength is acceptable for use in absorbency measurement.

Precautions for Use

- ✓ 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.
- ✓ 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 (NaN_3) 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.

Assay Protocol

Reagent Preparation

- ✓ All reagents should be brought to reach room temperature (25-28ºC) before use and all reconstitute tubes may take 30-40 minutes before completely dissolved.
- ✓ Dilute wash buffer, desire amount with distilled water (1 part with 19 parts). The buffer is stable for 1-3 months, if stored at 4-8ºC.
- ✓ Enzyme Conjugate, store at -20ºC for good stability, if not used immediately.
- ✓ Highly concentrated samples should be diluted wit sample diluent (eg. 1:5, or 1:10), to bring on to a readable range on the curve.

Assay Procedure

1. Pipette 25 ul of reconstituted standards.
2. Add 25 ul of samples into appropriate wells marked for further identification.
3. Add 100 ul of Cortisol Enzyme Conjugate Solution (after reconstitution). to each well.
4. Incubate for 1hours at 37℃.
5. Terminate the reaction and wash the plate 4-5 times with Wash Solution (250-300 ul) per well. Invert plate, tap firmly against absorbent paper to remove any residual moisture.
6. Add 100 ul 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 50ul of Stopping 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℃). For temperature outside this range, the duration of the incubation should be adjusted by approximately 1 minute/1℃.*

Data Analysis

Calculation of Results

1. Calculate the mean absorbance values (A) for each set of reference standards, controls, samples and blanks.
2. Subtract the value for blanks from those for standards, control and unknown samples.
3. Calculate the B/BO% values by dividing each value by the value for the zero-standard.
4. 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.
5. Using the graph read off the Cortisol concentrations for the unknown samples.
6. You may use any commercial assay soft-ware to analyze the data.

Performance Characteristics

- Sensitivity

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 1.0 ng/ml.

Resources

References

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Plate Layout

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