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# Product Information

## Sheep Cortisol ELISA kit

Catalog Number: EA100856
Storage Temperature: 2 – 8°C

# Instruction for Use

### Intended Use

The OriGene Cortisol ELISA kit is used for the quantitative measurement of Cortisol in sheep serum or plasma.

### Background

Cortisol (hydrocortisone, compound F) is the most potent glucocorticoid synthesized from cholesterol. Cortisol is found in the blood either as free Cortisol, or bound to corticosteroid-binding globulin (CBG). Cortisol production has an ACTH-dependent circadian rhythm with peak levels in the early morning and a nadir at night. The factors controlling this circadian rhythm are not completely defined. Serum levels are highest in the early morning and decrease throughout the day. In the metabolic aspect, Cortisol promotes gluconeogenesis, liver glycogen deposition, and the reduction of glucose utilization. Immunologically, Cortisol functions as an important anti-inflammatory, and plays a role in hypersensitivity, immunosuppression, and disease resistance. This kit can detect low level of cortisol in sheep serum or plasma (less than 0.1 ng/ml).

### Principle of the test

The Cortisol is a solid-phase competitive ELISA. The samples and Cortisol enzyme conjugate are added to the wells coated with anti-Cortisol monoclonal antibody. Cortisol in the patient's sample competes with a Cortisol enzyme conjugate for binding sites. Unbound Cortisol and Cortisol enzyme conjugate is washed off by washing buffer. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of Cortisol in the samples. A standard curve is prepared relating color intensity to the concentration of the Cortisol.

### Components

| MATERIALS PROVIDED | 96 Tests |
|---|---|
| 1. Microwells coated with Cortisol MAb | 12x8x1 |
| 2. Cortisol Standard: 7 vials ( ready to use) | 0.5 ml |
| 3. Enzyme Conjugate (20X) | 1.2 ml |
| 4. TMB Substrate: 1 bottle (ready to use) | 12 ml |
| 5. Stop Solution: 1 bottle (ready to use) | 12 ml |
| 6. 20X Wash concentrate: 1 bottle | 25 ml |
| 7. Assay Diluent | 24 ml |

## Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

## Disclaimer

This product is for research use only and not intended for diagnostic procedures.

## Specimen Collection Handling

1. Collect blood specimens and separate the serum immediately.
2. Plasma should be treated with EDTA.
3. Plasma samples may be stored at 2-8°C for up to 8 hours, and should be frozen at -20°C or lower for up to 4 months.
4. Avoid multiple freeze-thaw cycles.
5. Prior to assay, frozen sera should be completely thawed and mixed well.
6. Do not use grossly hemolyzed or lipemic specimens.

## Reagent Preparation

1. **Cortisol-enzyme Conjugate Solution**
   Dilute the Cortisol enzyme conjugate 1:21 with assay diluent in a suitable container. For example, dilute 100µl of conjugate with 2 ml of assay diluent buffer for 10 wells (a slight excess of solution is made).
2. **Wash Buffer**
   Prepare 1X Wash Buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

## Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

1. Place the desired number of coated strips into the holder
2. Pipet 40 µl of Cortisol standards, control and patient's saliva samples.
3. Add 200 µl of Cortisol Enzyme Conjugate to all wells.
4. Incubate for 60 minutes at room temperature (18-26°C) with shaking.
5. Remove liquid from all wells. Wash wells three times with 300 ml of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature with shaking.
8. Add 50 µl of stop solution to all wells.
9. Shake the plate gently to mix the solution.
10. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stop solution.

## Calculation of Results

The standard curve is constructed as follows:

1. Check Cortisol standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for Cortisol standards (Y-axis) versus Cortisol standard concentrations (X-axis). Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

## Example of Standard Curve

| | Conc. ng/mL | OD 450 nm |
|---|---|---|
| **Std 1** | 0 | 2.62 |
| **Std 2** | 1 | 2.37 |
| **Std 3** | 5 | 1.65 |
| **Std 4** | 10 | 1.24 |
| **Std 5** | 20 | 0.83 |
| **Std 6** | 40 | 0.59 |
| **Std 7** | 80 | 0.33 |

## References

1. Kabaroff L, Boermans H, Karrow NA: Changes in Ovine maternal temperature and serum Cortisol and interleukin-6 after challenge with E. coli LPS during pregnancy and early Lactation. J Anim Sci 84: 2083-2088, 2006.
2. Stewart PM, Seckl JR, Corrie J, Edwards CRW, Padfield PL: A rational approach for assessing the hypothalamo-pituitary-adrenal axis. Lancet 5:1208-1210, 1988.
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