REFERENCES

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CALBIOTECH

Sheep Cortisol ELISA

Catalog No. CO116S-400 (96 tests)

INTENDED USE

The Calbiotech Cotinine Direct ELISA Kit is intended for the measurement of Cotinine in Sheep serum or urine. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

Cortisol is the primary glucocorticoid in most mammals, regulating stress response, metabolism, and immune function. This assay detects cortisol in ovine samples for studies on hypothalamic–pituitary–adrenal (HPA) axis activity, livestock stress physiology, and environmental or nutritional influences on animal health.

PRINCIPLES OF THE TEST

The Cotinine kit is a solid phase competitive ELISA. The samples and Cotinine enzyme conjugate are added to the wells coated with anti-Cotinine antibody. Cotinine in the samples competes with a Cotinine enzyme (HRP) conjugate for binding sites. Unbound Cotinine and Cotinine enzyme conjugate is washed off by washing step. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of Cotinine in the samples. A standard curve is prepared relating color intensity to the concentration of the Cotinine.

MATERIALS PROVIDED	96 TESTS
1. Microwell coated with Cortisol MAb	12x8x1
2. Cortisol Standard: 7 vials (ready to use)	0.5 mL
3. Cortisol Controls: 2 vials (ready to use)	0.5 mL
4. Enzyme Conjugate (20X)	1.2 mL
5. Assay Diluent	24 mL
6. TMB Substrate: 1 bottle (ready to use)	12 mL
7. Stop Solution: 1 bottle (ready to use)	12 mL
8. 20X Wash Concentrate: 1 bottle	25 mL

MATERIALS 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

STORAGE AND STABILITY

1. Store the kit at 2 – 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

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WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

1. The standards contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

REAGENTS 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 2ml 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 (25ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature.
Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipette 40 µl of Cortisol standards, control and patient's sera.
3. Add 200µl of Cortisol Enzyme Conjugate to all wells.
4. Incubate for 60 minutes at room temperature (20-25°C) with shaking.
5. Remove liquid from all wells. Wash wells three times with 300 µl 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 (20-25°C) with shaking.
8. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 20 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 (vertical axis) versus Cortisol standard concentrations (horizontal axis) on a linear graph paper. 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 a standard curve

Standard	Conc. (ng/ml)	OD (450 nm)
1	0	2.62
2	1	2.37
3	5	1.65
4	10	1.24
5	20	0.83
6	40	0.59
7	80	0.33

LIMITATIONS OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.